flock or farm. The set-up of a TSE surveillance programme should be such that TSE positive results can be linked to the farm or flock of origin. If test positive animals are found, and depending on the prevalence rate observed, a complementary surveillance design could be targeted at farms in order to estimate the percentage of affected ruminants per affected farm.

C. Sample size, taking into account possible temporal and geographical variation in challenge.

If information is needed about TSE prevalence in different subgroups of the target population (sub-grouping by age or region, for instance) then separate sampling schemes need to be set up specifying, for each subgroup, the prevalence to be detected and necessary precision. For example, cattle born before versus after the full implementation of a feed-ban constitute two separate, important sub-populations to be considered separately for surveillance purposes.

D. Level of possible risk, if any, to consumers resulting from BSE in small ruminants.

With the currently available rapid tests (January 2003), BSE surveillance of adult ruminants has to proceed in two stages: rapid TSE testing to identify TSE positives, and a second form of testing [to be determined, but preferably shorter duration than transmission studies in mice[16]] used to discover if any TSE positives were in fact BSE positive. From the above table it can be derived that, to exclude a BSE prevalence in TSE rapid test positive adult sheep of 1 in 200 TSE test positives, a Member State would need to apply second-stage BSE testing to between 600 (95%) and 920 (99%) TSE rapid test positives without finding any TSE positive small ruminant which is BSE positive. To exclude BSE prevalence in TSE rapid test positive adult sheep of 1 in 2000 TSE test positives, a country would need to apply second-stage BSE testing to between 6000 (95%) and 9200 (99%) TSE rapid test positives.

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16 The SSC is currently preparing a specific opinion on this subject.

E. Genotyping of small ruminants

To enhance knowledge about susceptible and resistant genotypes per country and gradually to quantify the relation between genotype and TSE susceptibility in Europe, the SSC recommended that:

1. a random sub-sample of 500 from the first 100.000 routinely slaughtered native adult sheep which are subject to rapid TSE testing per country is genotyped.

2. every rapid TSE test positive adult animal is genotyped together with two set of three suitably sampled controls per TSE positive case

Countries which have not excluded that their TSE prevalence is 50 or more per 1 million adult sheep should continue rapid TSE surveillance until they have genotyped at least 100 TSE test positive adult sheep together with their associated controls.

F. Measures against diversion.

Even if the major target population consists of risk animals, the testing of healthy stock in parallel is recommended for at least the first year of active TSE surveillance for quality assurance in implementing the surveillance programme. Thereafter, active surveillance at slaughterhouses only needs to be sufficient to guard against diversion.

If the target population consists primarily of animals sent for routine slaughter (as may be the case for small ruminants) then escape routes, such as channelling of suspect animals for unmonitored disposal, should be controlled.

G. Quality assurance and reporting standards

1. **Practically oriented protocols** for random sampling from the target population should be properly documented and preferably peer-reviewed.

2. **Born After Real Ban (BARB)-controls study.** Any BSE positive, whether a clinical case or surveillance-detected, born after the start date of a Member State's total feed ban should be followed up; together with suitable controls.

3. **Reporting format** should differentiate:

   - clinical TSE cases from TSE test positive surveillance-detected animals

- imported from native animals

and should include: month and year of birth, cause of death , month and year of death, age at death, region of slaughter or death; TSE rapid test result and type of test used; and, for small ruminants, flock, farm and genotype.

4. **The whole survey system** should be subject to regular and formal quality assurance.

5. **Number of BSE cases adjusted for surveillance-coverage.** Comparisons between Member States, or between reporting years per Member State, should be based on the Member State's surveillance-adjusted BSE cases.

H. Surveillance in small ruminants

TSE surveillance

Scrapie in sheep is under-reported. When clinical scrapie is followed up by veterinary surveillance of the host flock or post-mortem testing, additional clinical or sub-clinical cases have been discovered in sheep with non-resistant genotypes.

If a) correction is made for under-reporting and b) it is assumed (conservatively) that there is at least one additional rapid TSE test positive adult sheep per scrapie case, then TSE prevalence in adult sheep could range from 20 to 500 TSE positives per 1 million adult sheep according to Member State. In practice, TSE surveillance in healthy adult sheep has revealed these prior estimates to have been indeed under-estimates.

By analogy with cattle, TSE prevalence may be substantially higher in fallen sheep than in similarly-aged sheep which are being slaughtered for human consumption. Because of their lower value, sheep are seldom sent for emergency slaughter. They may be killed on farm, or die where they roam, or be sent directly to a rendering plant or disposal site. Thus surveillance of risk sheep, is unlikely to be comprehensive. The target group of risk animals in small ruminants is therefore not comparable to the corresponding target group in cattle.

TSE surveillance in sheep and goats should with the currently available tests target the age-group in which TSE test positivity is most likely, probably adults.

Active rapid TSE test surveillance of native adult sheep at slaughterhouses is therefore proposed as the first step in improving scrapie surveillance. Escape routes should be controlled. Additional surveillance schemes for imported sheep may need to be considered.

Later stages of active TSE surveillance may be envisaged, as follows:

- surveillance based on rapid TSE testing in the spleen of sheep under 12 months which have been sent for slaughter, if suitable tests are available.

- surveillance based on flocks, because scrapie eradication policies are flock-based, and making use of genotyping and, potentially, tonsil-based TSE testing of live sheep to limit within-flock culling.

Table 1 provides the numbers of adult sheep brains for TSE detection according to likely prevalence & probability level for Member States whose national flock is under 1 million. Interval estimation of TSE prevalence rates with adequate precision, rather than scrapie detection, is likely to be the surveillance goal in most member states, however.

Relevant SSC opinion (see annex II): **88**

RISKS OF BSE IN PIGS

By G.A.H. Wells.

The recognition of bovine spongiform encephalopathy (BSE) in domestic cattle in the United Kingdom (UK) in 1986 inevitably led to concerns about the potential risk of similar diseases occurring in non-ruminant livestock or farmed food species. Research was quickly directed toward the investigation of the susceptibility of pigs to infection with the bovine agent. Investigations into processing and trading practices within the rendering and feedstuffs industries in the UK identified the fact that consumption of meat and bone meal must have led to significant exposure of pigs to the agent of BSE.

The ban on the use of ruminant protein in ruminant feed in the UK in July 1988 raised concern about inter-species recycling. Also in the UK, between 1990 and 1996, some feed companies stopped using animal proteins, other than fish meal and milk products, in feeds for pigs and poultry. Others continued to use these ingredients until the use of mammalian meat and bone meal in livestock feed was banned in 1996. Despite the 1996 ban in the UK, the feeding of mammalian meat and bone meal to pigs and poultry remained legal in other countries of the European Union (EU). Since January 2001 the use of all processed mammalian protein in feeds for farmed animals has been banned throughout the EU with periodic adjustments, but its use in pig and poultry feeds in other parts of the world continues.

Experimental studies of the transmissibility of BSE to pigs

Studies to test the transmissibility of the BSE agent to pigs began in the UK in 1989. Parenteral inoculation of the agent to 10 pigs, by three routes simultaneously, produced disease with an incubation period range of 69 -150 weeks. Pre-clinical spongiform encephalopathy was detected in two pigs killed 105-106 weeks post-inoculation (p.i.). Infectivity was detected by bioassay in inbred mice in the central nervous system (brain and spinal cord) of all pigs which developed spongiform encephalopathy. Infectivity was also found in the stomach, jejunum, distal ileum and pancreas but not in other tissues assayed (spleen, thymus, mesenteric lymph node, liver and kidney) of the terminally affected pigs. These findings show that pigs are susceptible to BSE and although infectivity was present in all the CNS tissues from exposed pigs that were tested, not all of the assay mice injected with brain from clinically-affected pigs developed the disease,

suggesting the existence of a species barrier to the transmission of BSE from pigs to mice which reduced the sensitivity of the bioassay. What was unexpected was the relatively few peripheral tissues in which any infectivity was detected. This finding again suggests that a large species barrier compromised the sensitivity of the bioassays.

In contrast to the transmission of BSE by parenteral inoculation, disease failed to occur in 10 pigs retained for seven years after exposure by feeding BSE affected brain on three separate days, at 1-2 week intervals. The amounts fed each day were equivalent to the maximum daily intake of meat and bone meal in rations for pigs aged eight weeks. No infectivity was found in tissues (brain, spinal cord, semitendinosus muscle, spleen, thymus, retropharyngeal, mesenteric and popliteal lymph nodes, stomach, distal ileum, pancreas, liver and kidney) assayed from the pigs exposed orally. It is suggested that these pigs did not become infected. That exposure of pigs to the BSE agent by feeding did not transmit the disease to pigs is in marked contrast to the now considerable body of evidence that BSE has transmitted, by natural or accidental means, via foodstuffs to several other animal species and to man and indeed has been transmitted by feeding BSE-affected brain tissue to several additional animal species.

Other studies make it likely that the effective exposure of pigs was further reduced by a species barrier to the oral transmission of BSE from cattle to pigs. The existence of such a barrier can be inferred from comparisons of the study findings with the results of an oral titration, in cattle, of a pool of 60 BSE-affected brain stems. All the calves exposed to the 100g dose of brain material developed clinical signs and histopathological lesions of BSE. The amount of the brain pool required to cause BSE in 50% of the exposed cattle is estimated to be less than 1g. The fact that none of the pigs appeared to become infected after being fed an average of 400 g of brain on each of three successive occasions (a total of 1,200 g) suggests the existence of a cattle-pig species barrier that reduced the effective oral exposure to BSE by as much as 100-fold, or even more.

The absence of a naturally occurring TSE cases in pigs

There have been no reports of a naturally occurring TSE in pigs in the United Kingdom even though in the period that cattle were being exposed to contaminated MBM, pigs were also being exposed. Moreover, the inclusion rates of MBM in commercial pig feeds were usually greater than in ruminant rations. It is difficult to estimate the degree of BSE contamination of MBM but approximations suggest that the experimental exposure to CNS tissue by feeding was 50,000 times more than the calculated exposure in the field.

The experimental exposure of pigs on just one of the three occasions was probably well in excess of the average life-time global exposure of pigs in the field to BSE.

If pigs were as susceptible to BSE by the dietary route as are cattle, with a similar median incubation and assuming that highest level of prevalence of infection was 1 per cent, then over 1,000 cases of BSE in pigs should have occurred by 2002.

The possibility of subclinical infection of pigs

It is possible that in the experimental exposure of pigs by feeding infection occurred but did not produce clinical or pathological evidence of disease and the mouse bioassay, across the pig-mouse species barrier, was too insensitive to detect infectivity in any of the tissues. But had primary infection of pigs from cattle with BSE occurred, there would have been the potential for recycling, as occurred in cattle, and, hence, amplification of a porcine-adapted BSE agent because of the inclusion in pig rations of MBM of porcine origin. Also, pig material contributed in greater proportion to MBM and therefore, any infection in pigs would have been transmitted to pigs with no species barrier effect and, had disease resulted, it might have been expected to occur with shorter incubation periods than primary foodborne transmission to pigs. The failure of recycling and amplification to produce clinical disease in pigs both before and, currently, six years after the end of such exposure, tends to negate the hypothesis of inapparent BSE infection in pigs. Experimental investigation of possible subclinical infection in pigs would require sub-passage of selected tissues, notably those of the alimentary tract, from the orally exposed pigs, employing the same species, or possibly transgenic mice expressing porcine PrP.

It can be concluded from the studies of the transmissibility of BSE to pigs that although pigs are susceptible to BSE when injected by combined parenteral routes, there is no evidence of transmission after exposure by feeding three doses of BSE-infected brain in amounts equivalent to the maximum daily intake of MBM formerly used in commercial pig rations. The simplest explanation of this finding is that the effective exposure of pigs by the oral route was insufficient to establish infection. These observations are in contrast to the susceptibility of cattle to oral infection with gram quantities of BSE-affected brain and to the major feedborne epidemic in the UK.

Present knowledge therefore does not provide scientific justification to include certain tissue of pigs in an SRM-ban.

Relevant SSC opinions (see annex II): **42**

RISKS OF BSE IN FISH.

By E.Vanopdenbosch

1. Introduction

Mammalian MBM and other mammalian products have historically been fed to farmed fish. Furthermore, intra-species and intra-order recycling via feed is common practice in fish farming. It was therefore important to address the question whether the latter practice could enable mammalian TSE agents to establish themselves in fish and for species adaptation of such agents to occur. This could lead to the development of a TSE in fish that might lead to a TSE epidemic in fish and/or create a health risk for the consumer. An assessment was made to advise whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSEs and, if appropriate, to suggest examples of conditions under which intra-species or intra-order recycling of fish could be allowed.

2. Relevant data and risk assessment

Feeding of farmed fish

The feeding with fishmeal raises the question of intra-species or intra-order recycling of fish tissues. Generally, although recycled fish in the form of fishmeal is the principal ingredient of feed for farmed fish, available information indicates that recycled farmed fish tissues are normally not used as an ingredient of fishmeal produced for fish feeds.

Research on TSEs in fish

The limited transmission studies that are currently in progress, i.e. the EC FAIR CT97 3308 project: "Separation, identification and characterisation of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish" have so far not provided evidence of TSE disease or infectivity replication in fish. However the possibility cannot be ruled out totally as PrP immune reactivity with an antibody that detects several mammalian PrPs has been reported in salmon (Gibbs and Bolis,1997) and Suzuki *et al.* (2002) found a candidate PrP-like gene in pufferfish *(Fugu rubripes)*, based on partial nucleic acid sequence homology.

However, Joly *et al.*(2002) concluded from their studies of PrP primary sequence that the PrP from fish is different from that in mammals and would be unlikely to share the pathological properties of mammalian PrP^{sc}. Both from the literature and from limited observations on fish, there is no evidence that TSEs would naturally exist in fish but the possibility cannot be totally excluded.

The risk of recycling of fish with regard to TSEs

Intra-species recycling could be regarded as more dangerous than producing feed for phylogenetically less related species, because of possible species barrier effects. However, in the absence of any data on species barrier effect in fish, the potential importance of intra-species recycling versus intra-order recycling cannot be estimated at present and neither are indications available that recycling in fish can be considered in the same context as is done for the domestic animal situation. Nevertheless, as long as the TSE problem is not relevant for fish and meat and bone meal from other possibly TSE infected species is not used as feed in aquaculture, recycling would not create an increased risk in respect to TSE in fish. The assessment would have to be reviewed, in line with the general principles of intra-species or intra-order recycling, if evidence is found of replication of TSE agent in fish.

The safest way for treating organic wastes of animal origin is processing at 133 °C under 3 bar steam pressure for at least 20 min. If this causes technological problems which might be expected with fish material, other time/temperature relationships may be applied but they have to be validated.

Possibilities of TSEs being recycled in fish

a. Wild fish

Many species of wild fish are carnivorous. There are two main scenarios that may result in a build-up of TSEs in wild fish.

Firstly, it is possible to hypothesise that a spontaneous TSE could develop in wild fish and that wild sea or river fish would have the capacity to recycle a TSE. However, is likely that natural predation would offer limited scope for amplification of the agent and the "infectivity" could remain confined to a small number of the sea or freshwater fish or mammals.

The second scenario involves direct exposure to TSE infected mammalian carcasses or their parts. Such an exposure could, as with the case of a spontaneous development of a fish TSE, initiate a cycle which could be propagated to other pelagic, demersal, freshwater (coarse or game) fish or marine of freshwater mammals. However, as for spontaneous development and under natural predation conditions, it is unlikely that significant amplification would occur among wild fish.

Dumping fish waste/offal at sea or in fresh water is likely to increase any theoretical possibility of recycling a TSE among wild fish as all ages, and sizes of fish could consume the waste.

b. Farmed fish

Farmed fish in general, need a protein source in their feed that originates from fish and is generally provided by a diet based on fishmeal. For this reason the possibility of recycling a TSE in farmed fish would be greater than is the case for wild fish.

To date, there is no evidence of a TSE in wild fish and therefore, no obvious possibility of "infected" wild fish being caught and processed into fishmeal. Likewise, although scavengers such as crustaceans or even marine mammals could also be infected, such fish or animals generally have a limited contribution to fishmeal. However, even a low-grade infection in the source fish could initiate a cycle in farmed fish if entire, or parts of, "infected" farmed fish were recycled without measures being taken to inactivate TSEs.

It is possible that without treatment to inactivate infectious prions, fishmeal and fish oil could transmit "infectious" prions to farmed fish. Intra-species recycling, due to the absence of a species barrier could increase the risk that TSE cases occur or undetected pools of infectivity develop. However, although intra-species recycling could be regarded as more dangerous than producing feed for phylogenetically less related species, because of possible species barrier effects, in the absence of any data on species barrier effect in fish, the potential importance of intra-species recycling versus intra-order recycling cannot be estimated at present and neither are indications available that recycling in fish can be considered in the same context as is done for the domestic animal situation.

Farmed fish could likewise be directly exposed to a mammalian TSE by direct exposure to an infected dead animal or its parts. This is an unlikely, but possible scenario.

3. Conclusions

Very little is known about the possible occurrence of TSEs in fish, but the possibility cannot be totally excluded. On the other hand, intra-species and intra-order "recycling" of fish materials occurs naturally in most if not all fish environments. It is therefore likely that natural predation would curtail amplification of any naturally occurring fish TSE agent. This principle may, however, not apply if the TSE agent were external to the fish environment/ecosystem and it is therefore justified to avoid the introduction of such agents to the fish environment, as this could possibly result in fish presenting a risk to other animal or human health vis-à-vis TSEs. It is further appropriate to highlight a number of additional uncertainties, such as the unknowns regarding the structure of putative fish PrP's, the level of the barrier in respect to intra-order recycling versus intra-species recycling, assuming that this is determined in fish by the PrP gene sequence, and the possibility that TSEs, if naturally present, may not manifest themselves in the same way as the known TSEs of mammalian species.

From the limited available research results, scientific literature on TSEs in fish and routine examinations of fish brain in the course of fish disease diagnosis, it can be concluded that there is no evidence that a natural TSE exists in fish and that there are no indications of replication of scrapie or BSE agent in experimental transmission studies.

On the question whether the feeding of wild fishmeal to farmed fish presents any risk to animal or human health vis-à-vis TSEs, it is therefore concluded that there is currently no evidence of any such risk existing although the data from the transmission experiments and from other sources are still very limited and incomplete.

Regarding the conditions under which intra-species or intra-order recycling of fish could be allowed, the following has to be considered:

- The risks caused by recycling in general, are addressed in the SSC opinion of 17 September 1999 on Intra-Species Recycling - the risk born by recycling animal

by-products as feed with regard to propagating TSE in non-ruminant farmed animals.

With regard to the specific TSE issue, some theoretical risks could exist, linked to feeding possibly TSE-contaminated feeds to animals currently believed to be not susceptible, including fish.

The possible TSE risks resulting from intra-species recycling of fish are therefore low if a number of conditions are complied with, as described in the SSC opinion of 22-23 July 1999 on Fallen stock, namely: safe sourcing [from an epidemiological point of view] with regard to the possible presence of TSE infectivity, of the material of origin: no fish should be recycled if it has been fed potentially contaminated mammalian MBM; appropriate treatment of the starting material

Relevant SSC opinions (see annex II): **41, 90, 91, 103, 104.**

BSE IN POULTRY (DOMESTIC FOWL OR CHICKENS)

BY: G. WELLS AND E. VANOPDENBOSCH

Concerns have occasionally been raised as to the theoretical risk that poultry could play a role in the exposure of, or the spread of TSEs, notably BSE, to humans by contracting the disease or by spreading the agent passively in excreted waste from consumed contaminated feed.

As far as these BSE risks in relation to birds are concerned, experimental data on the susceptibility of avian species are available only for the domestic fowl. They show that as yet there is no experimental evidence that BSE can be induced in this avian species by parenteral inoculations (which have included i/c injection), or oral challenge. Similarly, chickens inoculated i/v with the agent of transmissible mink encephalopathy (TME) did not develop disease but the agent could be recovered from their lymphoid tissues five months after they were inoculated. However, this persistence of agent could be explained as residual inoculum. The possibility of *active replication of agent* in birds is thus considered to be remote, if it occurs at all.

It has to be assumed that in the UK poultry were exposed (as were, for example, pigs) to high amounts of BSE-infectivity before their feeding with ruminant MBM was banned. Current disease monitoring systems are regarded to be unlikely to identify cases of TSEs in poultry, not least because of the short life-span of most commercially reared birds. However, higher incidence levels and shorter incubation periods, which could be anticipated with the occurrence of within-species re-cycling of agent, had poultry become infected, would probably not have gone unnoticed under all circumstances.

The possibility that poultry (as might be proposed for pigs or fish) to act, after oral challenge under field conditions, as healthy silent carriers in the spread of TSE-agents is still hypothetical and no results of experiments conducted as yet are available to support this hypothesis.

Birds may potentially ingest BSE infectious material[17] and *spread* ingested agent through the dissemination of faeces as it is unlikely that the pathological prion protein would be completely destroyed in the digestive tract. Moreover, plumage, claws and beak may also be contaminated with infectious material, which is then released into the environment.

If poultry are fed with animal-derived products that may contain BSE infectivity the following measures are considered to reduce such recycled infectivity:

- exposing the recycled animal material to a treatment by 133°/20'/3b or equivalent conditions,
- excluding those tissues known to carry the highest infectious load (ruminant Specified Risk Materials),
- excluding risk waste and fallen stock from the production of feed,

stop feeding poultry possibly contaminated feed for a sufficiently long period of time before slaughter in order to reduce the risk of recycling infectivity via the gut-content.

Relevant SSC opinions (see annex II): **43, 90, 91, 103, 104.**

[17] This may be via concentrate feed diets or, in the case of necrophagous and some omnivorous species of birds, through direct consumption of parts of infected bovine carcases or offals.

BSE IN SMALL RUMINANTS

by E Vanopdenbosch and G.A.H. Wells

Sheep and goats in many countries have probably been exposed to the BSE agent through MBM as a result of past feeding practices[18]. Because it has been experimentally demonstrated that BSE can be orally transmitted to certain genotypes of small ruminants, it should be assumed that BSE could have been introduced into the sheep and goat population. If the agent behaves like scrapie in these species it is possible that it has then been maintained, propagated and/or recycled by horizontal and vertical transmission[19]. Hence the risk could persist, even after effective implementation of the ruminant feed bans, which bans the feeding of ruminant meat-and-bone meal to small ruminants. At present however there is no evidence that BSE is present in small ruminants under field conditions and no indications pointing toward an increased likelihood of this being the case.

At present the Scientific Steering Committee (SSC) considers that the risk of BSE in small ruminant is "possible". Should the risk become "probable", current practices of safe sourcing of small ruminant materials by exclusion of certain Specified Risk Materials would no longer be adequate, but a more comprehensive approach for sourcing small ruminants materials would be needed. Such an approach should combine different strategies including removal of tissues known to pose a risk of infectivity from a given age, testing for BSE, genotyping and breeding for BSE-resistance, flock certification and individual animal and flock tracing.

To establish such a comprehensive approach, consideration would need to be given to the issues discussed below.

1. Distribution of infectivity in experimentally infected BSE-susceptible animals

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[18] The actual feeding practices of small ruminants, e.g., the age and extent of MBM feeding, are nonetheless different from cattle. They will also vary depending on whether the animals are to be used for meat, wool or dairy purposes.

The amounts, distribution and kinetics of accumulation of PrP^{Sc}, and by implication presumably BSE infectivity, differ in sheep experimentally infected with BSE by the oral route from those in cattle. Data indicate a widespread involvement of lymphoid tissues early in the incubation period. After one month from exposure to the BSE agent, susceptible sheep show an estimated significant load of BSE infectivity in the intestine, lymph nodes, tonsils, stomach and spleen. Data from experimental BSE in one sheep breed suggests that after 36 months of exposure the estimated total BSE infectivity load in the animal body is much higher and the distribution of infectivity very different. However other breeds may differ. When compared to the central nervous system tissues, the PrP^{Sc} load in the intestine of BSE-infected small ruminants is relatively higher at the beginning of the incubation period and of the same order of magnitude toward the end of the incubation.

The tissues/organs of BSE-infected susceptible small ruminants that, according to current knowledge, contain, or may contain BSE-infectivity are as follows: the head, the spinal cord and associated dorsal root ganglia, peripheral nervous tissues, the spleen, other lymphoid tissues (e.g. tonsils) and lymph nodes (e.g. prescapular lymph nodes and supra mammary lymph nodes), liver, pancreas, placenta, the alimentary tract from oesophagus to rectum, (i.e. not only the intestine but, the forestomachs and the abomasums and closely related lymph nodes, including the mesenteric lymph nodes and the mediastinal lymph nodes; also the innervation of the entire alimentary tract.

2. Scrapie and BSE- resistant and susceptible small ruminant genotypes

It has been demonstrated in experimental models of TSE diseases that the combination of the infecting strain of TSE agent and the genotype of the host PrP gene play a major role in determining relative incubation periods between model systems. Together these two factors also affect the targeting of infection to different organs and to different parts of the brain. The relative dose required to infect the host is also affected by these two factors.

The use of the words "susceptible" and "resistant" in what follows requires careful definition. They should be seen as relative terms in a continuum of susceptibility, not as absolute statements. By "more susceptible" it is implied that animals can be

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19 Maternal transmission is unproven in goats.

infected by a relatively small amount of infectivity, even by a relatively inefficient route (e.g. the oral route). By contrast "more resistant" implies that a larger dose of infective material is required to infect the animal and possibly by an efficient route (e.g. the intracerebral route). Although it is often the case that more susceptible models have relatively short incubation periods, susceptibility and resistance should not be confused with length of incubation period, since in some cases highly susceptible animals can have long incubation periods.

As far as the *genetic susceptibility of* ***sheep*** *to BSE* is concerned, sheep PrP genotypes and their effect on incubation period and pathogenesis are very complex and poorly understood. The available knowledge is based on a few published experiments carried out on small numbers of animals involving only a very small proportion of sheep breeds. Further studies are in progress. The results obtained indicate variation such that it is difficult, at present, to draw specific conclusions, or to make generalisation on host susceptibility to BSE in sheep. What follows should therefore be interpreted in this context.

Results to date have been interpreted that, in general, the relationship between PrP genotype and susceptibility is similar for scrapie and BSE in some breeds (e.g. in Suffolks). Susceptibility to these two TSEs is linked to PrP genotype, with codons 136, 154 and 171 being of major importance. In some breeds (e.g. Suffolks) sheep which are homozygous for glutamine (Q) at codon 171 are more susceptible to scrapie than other genotypes and can also succumb to experimental BSE. In other breeds e.g. Cheviots there other genotypes (those with Valine at codon 136 are more susceptible to natural scrapie. Nevertheless Cheviots with Alanine (A) at 136 have shorter BSE incubation periods. Available findings indicate that, after an exposure to a high dose of BSE-infectivity, detectable infection may be widespread in the lympho-reticular system a few months after exposure. Furthermore, in natural scrapie of Romney sheep (to which pathogenetically experimental BSE in sheep bears a resemblance), PrP^{Sc} can be detected from two months of age in Peyer's patches and mesenteric lymph nodes in the VRQ/VRQ genotype.

Available evidence indicates that sheep that are homozygous for the arginine (R) allele at codon 171 are the most resistant to development of the disease upon challenge with BSE-infected material. Infection of this genotype has been shown to occur after intracerebral infection but the development of the disease in these sheep,

if it occurs at all, would probably be slow and not result in significant infectivity levels in young animals.

Sheep that are heterozygous with one arginine (R) at codon 171 show an intermediate degree of resistance to BSE-infection and a distinct pathogenesis, as indicated by a different pattern in levels and distribution of infectivity in tissues and a much longer incubation period compared to that of genotypes which have a shorter incubation period. In consequence, for any given level of exposure to the BSE agent, the probability of finding clinical BSE or infectivity in tissues is lower in these sheep than in susceptible animals. Moreover, during the pre-clinical phase, PrP^{Sc} has not been detected, so far, in the enteric (autonomomic) nervous system of heterozygotic ARR/ARQ or ARR/VRQ sheep.

Until demonstrated otherwise in several models of sheep TSEs it must therefore be assumed, as a reasonable worst case, that after infection, there may be a rapid rise in the amount of infectivity in lymphoid and other peripheral organs of both susceptible and semi-resistant sheep genotypes but that resistant sheep may harbour less infectivity early in the incubation period.

3. Rapid tests to identify BSE-affected small ruminants

The currently available rapid post-mortem tests for detection of bovine BSE would certainly be useful to identify affected small ruminants. However, they would not offer the same degree of consumer protection as for bovines, because of the pattern of pathogenesis in BSE-susceptible small ruminants which may result in the presence of infectivity in peripheral tissues very early in the incubation period.

Tests for use on tissues that show infectivity in the early stages of incubation such as the lymphoid tissues are still being developed and will probably not be available for routine applications in the immediate future. Such tests would only permit an early identification of the infected susceptible small ruminants that pose a BSE risk, if sensitive enough to detect low levels of BSE-infectivity. On the other hand, only tests applied to CNS at the end of the incubation period are likely to be useful to detect BSE-affected semi-resistant sheep because detectable infectivity may be absent in certain lymphoid tissues of these genotypes.

4. Breeding for TSE resistance in small ruminants.

Since available data indicate that the relationship between sheep genotype and susceptibility to a TSE is similar for scrapie and BSE, breeding for scrapie resistant sheep is also expected to result in BSE-resistant sheep.

Breeding for resistant PrP genotypes is now being carried out in a number of countries, including the UK, the Netherlands and France. Concerns have been expressed about the potential long-term effects of such nation-wide and generalised programmes. These include the possible emergence of a TSE strain to which ARR sheep are highly susceptible, the possible deleterious effect of R171 on normal PrP function, and possible co-selection for negative traits. It may be expected that breeding for ARR/ARR genotypes in some breeds of sheep would be a multi-step process involving (a) ram genotyping scheme to increase frequency of the ARR allele in healthy flocks, (b) monitoring for scrapie on farms taking part in the programme and (c) dealing with scrapie affected flocks. Such a programme should initially be targeted at risk population or risk areas and would require:

- The availability of an acceptable method of identifying individual sheep (for example, electronic chips or boluses);

- For each important breed, an approximate knowledge of the frequency of ARR/ARR sheep to give an estimate of how quickly the breed would be able to move towards use of ARR/ARR rams only.

- An agreed procedure on scrapie monitoring, taking into account that very young animals or animals of heterozygous genotype may not show easily identifiable PrP^{Sc} in peripheral tissues.

- A programme of genotype monitoring of scrapie cases as recommended in the SSC's opinion of 30 November 2001 on Requirements for statistically authoritative BSE/TSE surveys, in order to have warning of the potential emergence of new scrapie strains able to cause disease more easily in the heterozygous genotypes (at the moment judged to be of intermediate susceptibility). Also the monitoring of the PrP^{Sc} profile will be needed in conjunction with strain typing.

- With respect to the occurrence of possible adverse effects, an effective monitoring of breed characteristics in scrapie resistant genotypes to obtain

reliable information on any undesirable changes (e.g. in birth weight, growth rates, strength and resistance to particular other diseases).

- Careful monitoring for comprehensiveness of protection against infection within the flock.

- Embryo storage for important pedigree flocks should be considered to protect against loss of important genetic traits.

5. **Flock certification**

Animals from a certified "Scrapie/BSE-free" (or preferably: "Scrapie/BSE-negligible risk") flock would represent no risk. However, infectivity can be present for years in animals and flocks that were apparently TSE-free in terms of clinical manifestation before coming under observation. The implementation of a comprehensive programme leading to the possible certification of flocks would therefore, in most cases and for most countries require many years. An approach of less stringent "provisional certification", is a possible alternative in the short term if, where necessary, it is applied in combination with other criteria such as testing and genotyping. The Section on TSE certification of bovine herds and small ruminant flocks (Part II.B) by E.Vanopdenbosch provides details on possible approaches to flock certification, which apply to both sheep and goats.

6. **Culling strategies**

Because of the transmissibility of the infection within a flock and between flocks by direct or indirect contacts, the elimination of the index case only will not eliminate the enhanced risk in a flock (of sheep and/or goats) where a clinical or sub-clinical TSE case has been confirmed. Therefore, a culling strategy could be considered which covers the entire flock where the index case was found and, in the case BSE was confirmed, the flocks that were in contact with the original flock via other small ruminants[20] or via grazing areas. Such culling would, however, have little or very

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20 Including via the offspring of the case

little risk reducing effect for sheep of the ARR/ARR genotype[21] or if the risk of transmission to other flocks was negligible.

The assessment whether the risk for transmission to other flocks was negligible would require that the animals introduced into a flock are identifiable and their history traceable and that they are genotyped. The risk would, for example, be negligible in the case of contacts with or imports from flocks certified to represent a negligible risk, if the contact only concerned the use of breeding rams (as compared to pregnant ewes) or if the imported animals tested negative with a validated in vivo test (once available).

Much of the above described approach will have to depend on the availability of detailed records and identification, and it may be impossible to trace sheep that have moved out of a flock or cohort historically, or indeed, to identify of cohort and offspring. If no tracing of animals exported from a BSE infected flock is possible other approaches (e.g. ad hoc epidemiological investigations) could be helpful to identify the potentially exposed flocks.

Whole flock slaughter might also turn out to be counter-productive for various scientific, economic and social reasons (e.g. it encourages even more under-reporting). A more effective policy is to identify infected animals as soon as possible and therefore remove them from the flock combined with a programme of genetic selection for resistant PrP genotypes. The development of an antemortem diagnostic test would facilitate this policy[22].

7. Geographical sourcing of small ruminant materials

The possible risk of materials sourced from small ruminants potentially being infected with BSE is likely to change with the geographical origin of the animals, depending on factors such as possible local unsafe feeding practices, possible episodic imports of BSE-affected animals and differences in the reliability of the

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21 Rapid TSE testing at slaughter of the spleen or brain of ARR/ARR animals above the age of 18 months from flocks with TSE would gradually provide conclusive evidence / reduce to negligible the risk that this genotype is a carrier of detectable infectivity levels.

22 Woolhouse, M. E., Stringer, S. M., Matthews, L., Hunter, N. & Anderson, R. M. (1998). Epidemiology and control of scrapie within a sheep flock. *Proc R Soc Lond B Biol Sci* **265**, 1205-10.

existing surveillance system. The **section on the Geographical BSE risk in Part II.B** elaborates further on this aspect.

Note on TSEs in goats.

Much less is known on TSEs is goats than on TSEs in sheep. In terms of risk management, the approach taken is to consider the conclusions for sheep as applicable to goats as well, at least until sufficient evidence will have become available to underbuild a possible goat-specific approach. The following evidence may nevertheless be mentioned:

- Scrapie occurs less frequently in goats;
- Maternal transmission has not been confirmed as occurring in goats ;
- Perhaps all goats may be susceptible to BSE or scrapie by the oral route under certain conditions. It is noted that the dimorphism in codon 142 of the caprine PrP gene appears to be associated with different incubation periods in goats experimentally infected with BSE or scrapie. Recent research has shown that goats have similarly complex PrP genetics as sheep. However, the relationships between breed, PrP polymorphisms and susceptibility to scrapie are not yet as well understood as in sheep and therefore a breeding programme towards TSE resistance in goats is not feasible on the basis of the present knowledge.

Relevant SSC opinions (see annex II): **30, 31, 32, 33, 34, 35, 36, 37, 38.**

PART II B

BSE RISK REDUCTION STRATEGIES

OVERVIEW OF THE APPROACH FOR THE GEOGRAPHICAL BSE RISK ASSESSMENT OF BSE IN BOVINES AND IN SHEEP

By V. Silano

The geographical BSE risk of BSE in cattle

1. Definitions

The Geographical BSE-Risk in cattle (GBR-C) is a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, pre-clinically as well as clinically, at a given point in time, in a country. Where presence of disease is confirmed, the GBR-C gives an indication of the level of infection as specified in the table below.

Table 1: Definition of geographical BSE risk (GBR-C) and its levels

GBR level	Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country
I	Highly unlikely
II	Unlikely but not excluded
III	Likely but note confirmed or confirmed, at a lower level
IV	Confirmed, at a higher level

2. Underlying hypothesis

The SSC-methodology for the assessment of the GBR-C is based on the assumption that BSE arose in the United Kingdom (UK) and was propagated through the recycling of bovine tissues into animal feed. Later the export of infected animals and infected feed provided the means for the spread of the BSE-agent to other countries where it was again recycled and propagated via the feed chain.

For all countries other than the UK, import of contaminated feed or infected animals is the only possible initial source of BSE that is taken into account. Potential sources such as a spontaneous occurrence of BSE at very low frequency or the transformation into BSE of other (animal) TSEs (scrapie, CWD, TME, FSE) arising in a country are not considered, as they are entirely hypothetical events. Blood, semen and embryos are not seen to be effective transmission vectors[23]. Accordingly, blood-meal is not taken into account, neither.

Cross-contamination of feed can be a way of propagating the disease. However, the influence of cross-contamination on the GBR-C has to be considered in the light of the risk that the animal protein under consideration could carry BSE-infectivity.

The possible impact of maternal transmission on the GBR-C has not been taken into account in this methodology because its occurrence is unconfirmed and its potential minor role in comparison to feed, also the qualitative nature of the GBR exercise. Similarly no "third route of transmission" was taken into account.

3. Information factors and model of the BSE cattle system

The methodology is based on 8 factors that were originally identified by the SSC in January 1998 as the most relevant information for carrying out the assessment (see **Table 1 and Figure 1**).

In order to clarify the often-protracted interaction between these factors, the SSC has adopted a simplified qualitative model of the cattle/BSE system (**Figure 1**) which focuses on a feed-back loop that is required to be activated to initiate a BSE-epidemic. This feed-back loop consists essentially of the processing via rendering of (parts of) cattle that carry the BSE-agent into feed and then the feeding of this contaminated product to cattle which then become infected and amplify the BSE-agent.

This feed-back loop is influenced by a number of factors that, on the one hand, may activate the loop and, on the other hand, might prevent this activation or slow down or reverse the build-up of BSE-infectivity within the system.

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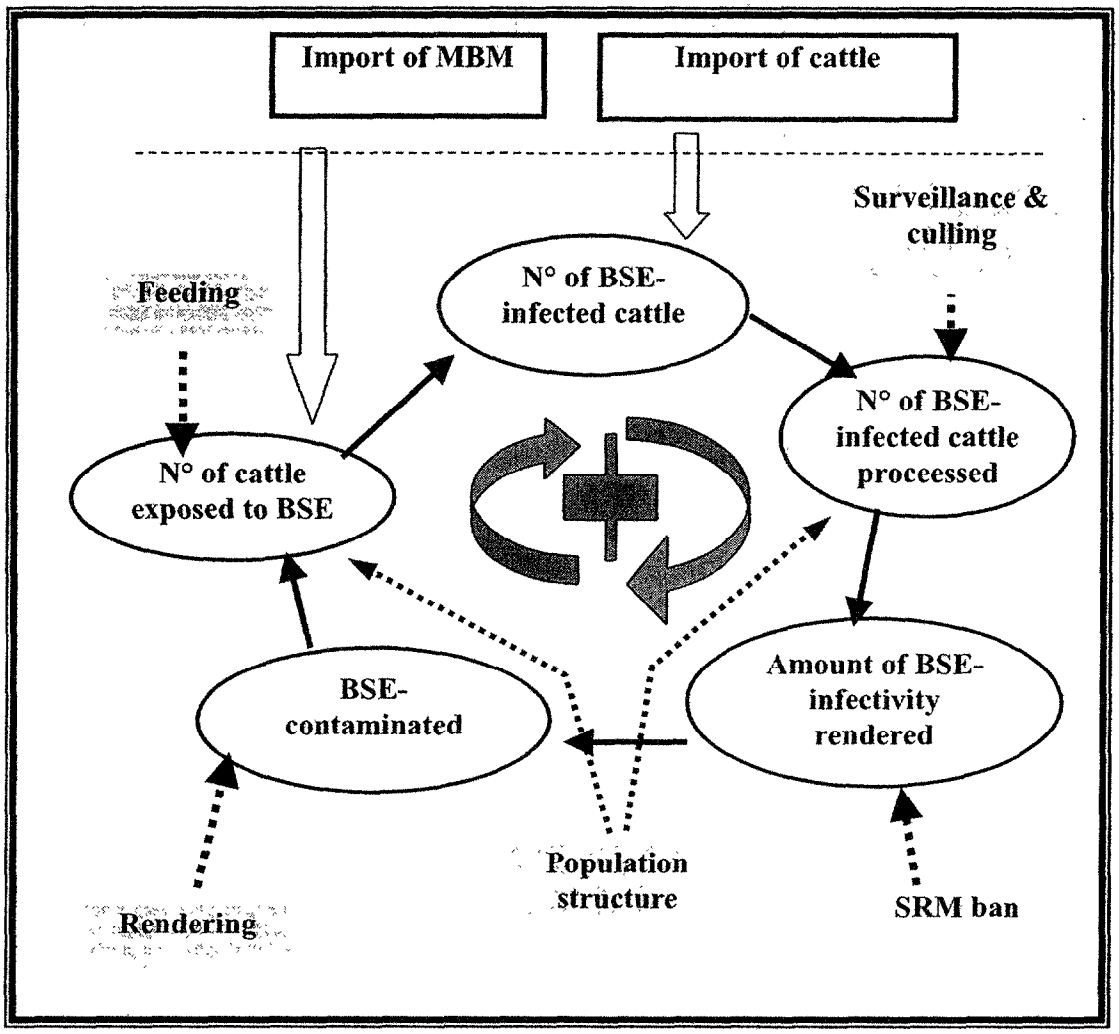
23 See SSC-opinion on vertical transmission, 18-19 March 1999 and on the safety of ruminant blood (13/14 April 2000)

Table 2: Information factors for assessing the GBR-C

Structure and dynamics of the bovine population
– Number and age distribution of beef and dairy cattle, both alive and slaughtered
– Husbandry systems, proportional to the total cattle population.
Surveillance of BSE
Measures in place to ensure detection of BSE-cases:
– Identification system and its tracing capacity
– Date since when BSE is compulsory notifiable and criteria for a BSE-suspect
– Awareness training (when, how, who was trained)
– Compensation (since when, how much in relation to market value, payment conditions)
– Other measures taken to ensure notification of BSE suspects
– Specific BSE-surveillance programs and actions
– Methods and procedures (sampling and laboratory procedures) used for the confirmation of BSE-cases
Results of BSE-surveillance:
– Number of cattle, by origin (domestic/imported), type (beef/dairy), age, method used to confirm the diagnosis and reason why the animal was examined (CNS, BSE-suspect, BSE-related culling, other)
– Incidence of reported BSE-cases by year, by birth cohort, and – if possible – type of cattle
BSE related culling
– Culling schemes, date of introduction & criteria used to identify animals that are to be culled
– Information on animals already culled in the context of BSE

Import of Cattle and meat-and-bone meal (MBM) – Imports of live cattle and/or MBM from UK and other BSE-affected countries – Information that could influence the risk of imports to carry the BSE agent (BSE-status of the herds of origin of imported cattle, precise definition of the imported animal protein, etc.) – Main imports of live cattle and/or MBM from other countries – Use made of the imported cattle or MBM
Feeding – Domestic production of MBM and use of MBM (domestic and imported) – Domestic production of composite animal feed and its use – Potential for cross-contamination of feed; measures to reduce and control it, results of the controls
MBM-bans – Dates of introduction and scope (type of animal protein banned for the use in feed in different species, exceptions, etc.) – Measures taken to ensure and to control compliance – Methods and results of compliance control
SRM-bans (SRM: Specified Risk Material) – Dates of introduction and scope; – Measures taken to ensure and to control compliance – Methods and results of compliance control
Rendering – Raw material used (type; annual amounts by type) – Process conditions applied and their share of the annual total domestic production.

Figure 1: The model of the BSE/cattle system used by the SSC



In the model used by the SSC the initial introduction of the BSE-agent has to come from outside the country under assessment – it is therefore called an external challenge of the system. For the UK it is assumed that the initial introduction of the agent happened before the period taken into account in this model. Two possible routes of introduction are considered: import of infected cattle or import of contaminated MBM. The factors assumed to be able to prevent the building-up of BSE-infectivity in the system are the following:

- Surveillance and culling;
- SRM-removal; exclusion of fallen stock;
- Appropriate rendering and processing methods;
- Appropriate feed bans.

In summary, the model can be basically broken down into two parts relating to challenge and stability, and the model assumes a mechanism for their interaction. "External challenge" refers to both the likelihood and the amount of the BSE agent entering into a defined geographical area in a given time period through infected cattle or MBM. "Stability" is defined as the ability of a BSE/cattle system to prevent the introduction and to reduce the spread of the BSE agent within its borders. Stability relies on the avoidance of processing via rendering of infected cattle and the avoidance of recycling of the BSE agent via the feed chain. A "stable" system would eliminate BSE over time; an "unstable" system would amplify it.

4. **Main results obtained**

The usefulness of the methodology is multiple. Firstly, it allows prediction of presence of BSE in the bovine population long before BSE-infected animals are discovered through passive *ad hoc* surveillance programmes; so far this has proved the case for several countries (i.e. Czech Republic, Germany, Italy, Slovakia, Slovenia, Poland and Israel). Secondly, it allows the understanding of the weaknesses of any specific country's system with respect to BSE and therefore is an effective guidance for the identification of the additional control measures needed to prevent BSE from entering the country and being amplified. Thirdly, the work carried out so far has produced the most powerful world-wide data basis on ruminant husbandry and feeding and on animal waste recycling and disposal. If properly exploited, this database is likely to prove to be very helpful for the control of other ruminant diseases and of other animal health problems. However, the most relevant implication in public health terms of the GBR methodology is the translation of this scientific evaluation into BSE safety criteria for a number of ruminant-derived products in different countries (See **Part II.C** on Safety of Products).

The GBR level of the countries that have been assessed so far by the Scientific Steering Committee is as shown in **Table 3**[24]:

Table 3: GBR status of 60 countries. The GBR methodology has also provided for each country an evaluation of the expected development (trend) of the GBR with time.

N°	Country name	Current GBR	Remarks
1.	Andorra	III	
2.	Albania	III	Re-assessment ongoing
3.	Argentina	I	
4.	Australia	I	Re-assessment ongoing
5.	Austria	III	
6.	Bellarus	III	
7.	Belgium	III	
8.	Botswana	I	Re-assessment ongoing
9.	Brazil	I	
10.	Bulgaria	III	
11.	Canada	II	Re-assessment ongoing; own risk assessment provided
12.	Chile	I	

24 These assessments can be found at: http://europa.eu.int/comm/food/fs/sc/ssc/index_en.html.

N°	Country name	Current GBR	Remarks
13.	Colombia	II	Re-assessment ongoing
14.	Costa Rica	I	
15.	Croatia	III	
16.	Cyprus	III	
17.	Czech Republic	III	Re-assessment ongoing;
18.	Denmark	III	
19.	El Salvador	I	Re-assessment ongoing
20.	Estonia	III	
21.	Finland	III	
22.	Former Yugoslavian Republic of Macedonia	III	
23.	France	III	
24.	Germany	III	
25.	Greece	III	
26.	Hungary	III	Re-assessment ongoing
27.	Iceland	I	
28.	India	II	Re-assessment ongoing
29.	Ireland	III	

N°	Country name	Current GBR	Remarks
30.	Israel	III	
31.	Italy	III	
32.	Kenya	II	Re-assessment ongoing
33.	Latvia	III	
34.	Lithuania	III	
35.	Luxembourg	III	
36.	Malta	III	
37.	Mauritius	II	
38.	Namibia	I	Re-assessment ongoing
39.	Netherlands	III	
40.	New Caledonia	I	
41.	New Zealand	I	
42.	Nicaragua	I	Re-assessment ongoing
43.	Nigeria	II	
44.	Norway	I	Re-assessment ongoing
45.	Pakistan	II	Re-assessment ongoing
46.	Panama	I	Re-assessment ongoing

N°	Country name	Current GBR	Remarks
47.	Paraguay	I	
48.	Poland	III	Re-assessment ongoing;
49.	Portugal	IV	
50.	Romania	III	
51.	San Marino	III	
52.	Singapore	I	
53.	Slovak Republic	III	Re-assessment ongoing;
54.	Slovenia	III	
55.	Spain	III	
56.	Swaziland	I	Re-assessment ongoing
57.	Sweden	II	Re-assessment ongoing
58.	Switzerland	III	Re-assessment ongoing
59.	Turkey	III	
60.	United Kingdom	IV	
61.	Uruguay	I	
62.	USA	II	Re-assessment ongoing; own risk assessment provided.
63.	Vanuatu	I	

THE GEOGRAPHICAL BSE RISK OF BSE IN SMALL RUMINANTS

BSE in sheep has not been proven under field conditions, but information obtained so far can be used as a scientific plausible stepping-stone for a hypothetical model for the occurrence and spread of a BSE epidemic in small ruminants, if indeed it did occur. This model for hypothetical BSE in sheep, combined with the experiences from the assessment of the geographical BSE risk in cattle, has led to the framework for assessing the geographical BSE risk in sheep and goats (GBR-S) described in the report adopted on 8 November 2002.

1. Definitions and methodology

A stepwise approach was developed to assess the geographical BSE-risk for sheep and goats (GBR-S) based on the exploitation of the geographical BSE-risk for cattle (GBR-C) in order to make possible public health decisions while the very time consuming tests now being proposed for the discrimination of BSE from scrapie are carried out.

For sheep, the same classification of GBR already in use for cattle, i.e. Levels from I to IV with exactly the same definitions after substitution of the word "cattle" with the word "sheep" is used. It acknowledges the peculiarities of sheep, as compared to cattle, concerning:

(a) **routes of infection** (not only contaminated feed, but also direct and indirect contact);

(b) **prevalence of BSE in a sheep scrapie population** (upperbound BSE prevalence assumed to be 1%);

(c) **prevalence of scrapie in small ruminants** (as a reasonable worst case hypothesis it is assumed a prevalence of 0.5% of scrapie in small ruminants);

(d) **prevalence of BSE in small ruminants** (assumed to be 0.05% as a maximum).

(e) **information factors and model of the BSE sheep system** The methodology proposed to assess the GBR-S is a stepwise systematic process that follows steps:

Step one - Countries in GBR-C levels III and IV

Based on the above mentioned assumptions, it is concluded that countries with GBR-C levels III or IV should be classified, even in the absence of notified BSE or scrapie-cases among small ruminants, into GBR-S level III unless data can be provided showing that, since 1980, it was very unlikely or unlikely that significant levels of potentially-infected MBM reached small ruminants through the feed chain. The methodology for assessing the data provided by a country to show that small ruminants were not exposed to significant levels of potentially-infected MBM since 1980 would be the one already developed for cattle by making use of the available information highlighted in **Table 4**. It should be understood that it would be extremely unlikely that such data would be available for most countries and that, in practice, classification in the level III GBR-S would be the most common and logical consequence for all these countries.

Table 4: Information elements for assessing the GBR for sheep and goats(a)

Structure and dynamics of the ovine/caprine population
- Number and age distribution of sheep and goats, both alive and slaughtered
- Information on husbandry systems used for sheep and goats
• type of main product: wool/meat/milk,
• intensive/extensive,
• productivity of milk-sheep/goats,
• co-farming of pig/poultry/cattle with sheep/goats,
• geographical distribution of sheep/goats, cattle and pig/poultry populations,
• size distribution of sheep flocks and goat herds,
• Internal animal trade: (n° and age distribution of sheep/goats annually traded

between flocks/herds, and between different husbandry systems and/or between different regions of the country.

Surveillance of TSEs in small ruminants

Measures in place to ensure detection of TSE (scrapie)-cases:

- Identification system and its tracing capacity (for sheep and goats)
- Date since when TSEs are (scrapie is) compulsory notifiable and criteria for a TSE (scrapie)-suspect
- Awareness training with regard to TSEs (scrapie) in small ruminants (when, how, who was trained)
- Compensation for animals culled in the context of scrapie eradication (since when, how much in relation to market value, payment conditions)
- Other measures taken to ensure notification of scrapie suspects
- Specific TSE/scrapie-surveillance programs and actions (detailed description, plans)
- Methods and procedures (sampling and laboratory procedures) used for the confirmation of TSE-cases

Results of TSE/scrapie-surveillance:

- Number of examined sheep and goats, by origin (domestic/imported), type (wool/milk/meat), age, method used to confirm the diagnosis and reason why the animal was examined (CNS, TSE-suspect, TSE-related culling, other)
- Result of the surveillance efforts
- Incidence of reported TSE-cases/n° of newly infected flocks by year of confirmation, by birth cohort of the confirmed cases, and – if possible – type of use (wool/meat/milk).

TSE related culling

- Eradication measures, including culling schemes, date of introduction & criteria used to identify animals that are to be culled

- Information on animals already culled in the context of TSE
Import/export of live animals (bovine/ovine/caprine) and of MBM (<u>Note:</u> Blood, semen, embryos or ova not seen as an effective transmission route. MBM is used as proxy for mammalian protein (other than milk) as animal feed)
- Imports/export of live animals (cattle/sheep/goats and/or MBM from/to UK, from/to other BSE-affected countries[25] and from/to other "BSE-free" countries; provide annual data per partner-country)
- Information that could influence the risk of imported live animals or MBM to carry the BSE agent (BSE-status of the herds/flocks of origin of imported cattle/sheep/goats, precise definition of the imported animal protein, information on the process conditions and raw material used for imported MBM, etc.)
- Use made of the imported animals and of the imported MBM.

[25] BSE-affected countries are all countries with confirmed BSE-cases and all countries classified by the SSC as GBR III, even if they have not notified any cases.

Feeding and cross-contamination

- Composition of the feed for ruminants (for cattle/sheep/goats give the percentage of grass/pasture, roughage, industrial feeds, protein concentrates used in on-farm preparation of compound feed for ruminants, feed additives, ... per species) and measures taken to control this composition
- Use of MBM (domestic and imported: for farmed animals (ruminant/non-ruminant), in pet food, fertilizer, or in other uses (please specify); information on how this use was controlled)
- Domestic production of composite animal feed and its use (type of feed mills (single line/multiple line plants, single/multiple species production), annual production of feed by target species and by feed mill, information on how the use of the produced feed was controlled).
- Potential for cross-contamination of feed for ruminants with MBM or blood during feed production, during transport and on-farm,

- measures taken to reduce it (labelling, awareness raising, technical installations);
- measures taken to control it (feed sampling (specify n° of samples taken from compound feed for ruminants per year and species, method of examination, place of sampling (feed mills, during transport, on-farm), other controls in feed mills, during transport or on-farm);
- results of the controls, handling of breaches.

Step one - Countries in GBR-C level I and II

To assess the GBR-S of countries with GBR-C levels I or II, it would be necessary to check that the challenge deriving from potentially-BSE-infected materials, already assessed for cattle as being negligible or very low, remains as such even after consideration of the additional challenge for the feed chain that might have occurred since 1980 through live sheep imported from BSE risk countries (this import, in fact, might have given origin to an internal production of potentially-infected MBM which could have reached both small ruminants and cattle). Should the challenge through the feed chain due to live small ruminants be found to be negligible throughout, the GBR-S classification would remain identical to the GBR-C classification? Otherwise the combined external challenge should be assessed and a

stability analysis conducted for the sheep feeding system since 1980, resulting most probably in a higher GBR-S level. The issue depends crucially on the stability of the system with the exclusion of any possibility that BSE infectivity can contaminate the feeding systems for small ruminants.

In order to apply to small ruminants the methodology already developed for cattle, one could use the same external challenge categories in use in the GBR-C, taking advantage of the available information on the imports of live small ruminants (this is potentially very important as the EUROSTAT data reveal very large number of animals being traded every year) from BSE-risk countries and on the reasonable worst case assumption for the prevalence of BSE in small ruminants. The data reported in **Table 5** should be fed as applicable to the GBR model and examined consequently.

Table 5. Level of external challenge resulting from import of live small ruminants from the UK or other BSE-risk countries.

Level of external challenge	Live small ruminants from the UK, 1988 – 1993	UK	Other countries
Extremely High	>10.000.000	**UK-imports before 88 and 94-97: *10; after 97: *100**	**Imports from other countries with a BSE risk: R1*1000, R2* 100**
Very High	1.000.000-<10.000.000		
High	100.000-<1.000.000		
Moderate	20.000-<100.000		
Low	10.000-<20.000		
Very low	5.000-<10.000		
Negligible	0-<5.000		

Step two

For countries that at the end of step one remain classified as GBRS level I or II, it would be necessary to estimate whether BSE might have entered the country through live small ruminants and transmitted through horizontal or vertical routes. To this end, use should be made of, the information when available on the numbers of imported live small ruminants from BSE-risk country and dates. The intended use of these animals is important because it is expected that a substantial proportion of these animals are scheduled for slaughter, but experience suggests that an appreciable proportion of the animals imported into one country may be rapidly

exported to another country. This will reduce the risk in the first country, but amplify the potential spread of BSE infectivity.

In order to develop different challenge levels for the horizontal transmission of BSE in small ruminants, it could be considered, as a starting point, that information derived from scrapie indicates that even a small number of infected sheep (according to a worst case hypothesis, even 1 animal can be at the origin of disease spread within a flock) is sufficient to generate and sustain an epidemic and that such a probability increases with the number of potentially–infected animals imported. This evaluation should be based on the same prevalence factor reported above. Therefore, significant probability of a of BSE epidemic in small ruminants would be associated for example with the import into a given flock of a few thousands breeding or milking sheep, whereas sheep imported for immediate slaughter would not be expected to make any major contribution to the risk.

The SSC stresses that this GBR-S model will need adjustments if or when new scientific data regarding probable/possible presence of BSE in small ruminants under field conditions become available, but supports the further development (and its application) of the present model if an acute situation concerning discovery of BSE in sheep under field conditions would occur.

Relevant SSC opinions (see annex II): **118 to 260**

PREVENTING RECYCLING OF INFECTIVITY: CULLING STRATEGIES

By D. Heim

Impact of culling

The probability that "at risk animals" epidemiologically linked to BSE-index cases are infected with BSE is somewhat higher than for the rest of the healthy cattle population. Culling therefore can avoid that some potentially infected animals enter the human food and animal feed chains and can therefore reduce the risk for humans and animals.

However, the impact of BSE-culling on the current pre-clinical BSE-incidence and the future clinical BSE-incidence is dependent on many factors and cannot easily be assessed.

Assumptions

Bovine Spongiform Encephalopathy (BSE) is not transmitted horizontally and the only significant transmission route is feed; it is hence not comparable to contagious diseases.

It is assumed that the majority of infections normally take place in the first months of life of calves.

As the incubation period of BSE is between 2 and 14 years (mean 60 months) with the vast majority of clinical cases being 4-6 years at clinical onset, the exposure event that lead to the development of a clinical case must have taken place 4-6 years previously for the majority of animals.

Current diagnostic tools do not allow the identification of animals in the early phases of the incubating period. The available methods (PrP^{Sc} detection) are able to detect a proportion of BSE a-symptomatic infected animals, not previously identified as suspects, i.e. a-symptomatic animals, but only then in the late stages of the incubation period.

BSE is a rare event. With the exception of the UK in the years of the height of the epidemic, the yearly incidence remained below 0,1 % (1.000/million) of the adult (>24 months) cattle population.

Factors that influence the efficiency of a culling policy.

The efficiency of any culling scheme is critically depending on the ratio of identified number of BSE-cases to the real number of cases. It seems logical that the willingness of farmers to notify a suspect case is influenced by the impact that this would have on his farm. Culling of a-symptomatic animals will make the impact more severe and less easily acceptable. A herd culling policy can be assumed to be a greater disincentive to notify a suspect than a birth-cohort culling.

On the other hand, culling only parts of the herd could be economically problematic for some farmers, e.g. If the industry denies taking milk or meat from herds where BSE has occurred.

Appropriate compensation schemes may buffer the impact of the culling scheme on the notification of BSE-suspects to some extent. The acceptance of any culling scheme depends on its assumed cost/benefit ratio. The cost depend mainly on the number of culled animals, the compensation paid, and the cost of collecting, culling, testing and disposing of these animals. The benefits of culling are determined by its "hit-rate": number of incubating animals per total number of culled animals, and hence the reduction of the current prevalence and of the future clinical cases saved per number of animals culled.

The public will not accept a culling scheme unless convincing and sound evaluation is provided of the efficiency of different culling schemes with regard to preventing a BSE-epidemic and reducing the risk for man.

The SSC has examined data from Switzerland, Ireland, Belgium, France and Portugal and theoretical back-calculations from the UK. The available data shows that the vast majority of the additional cases found in the population of cattle that were culled under the applied (herd-) culling-scheme, while not showing signs of BSE, fell indeed into the birth-cohorts as defined above. In the second SSC-opinion it was confirmed that data from France, Germany, Portugal, Spain and Ireland showed that all secondary cases found when testing animals culled under the herd culling strategy belonged to the birth cohort of the index cases.

Recommendations

Ideally, all cattle exposed to the same feed as the index case should be culled but this target population may be difficult to be identified.

The limited available information indicates that herd culling is already having some effect both in terms of eliminating otherwise not identified (pre-clinical) cases and in terms of preventing future cases.

However, the data also indicate that largely the same effect can be reached by birth cohort (see definition below) culling, i.e. only culling about 1/3 of the animals that are culled under a herd-culling scheme.

In view of the limited data available, the impact of the epidemiological situation in a country on the relative efficiency of practically possible culling schemes cannot be fully assessed. It is, however, likely that birth cohort culling is in most cases the more cost-efficient approach.

The SSC recommends the application of birth-cohort culling whenever a domestic index case appears, irrespective of the prevailing epidemiological situation and has stated that cohort culling is apparently as effective as herd culling. All animals from these cohorts should be traced, killed and destroyed, independent of their current localisation.

This position is based on the definition of a birth cohort including all animals born and/or raised in the same herd as the confirmed case within approximately 12 months before and after the date of birth of the index case.

The SSC further recommends that all members of these birth cohorts that are older than 24 months are systematically examined for the presence of PrP^{Sc} in their brain or spinal cord using a validated method.

Relevant SSC opinions (see annex II): **76, 77, 83.**

By Emmanuel Vanopdenbosch

TSE certification of bovine herds

1. Terms of reference and scope

In the medical, pharmaceutical sector, one of the preconditions for putting animal derived products on the market is that they are derived from safe sources. Safe sources might be countries, accepted to be BSE-free. For countries, which may have or have had BSE at some point in time, the practical concept of "negligible BSE-risk" herds, sometimes called "closed herds" needed to be developed and the SSC addressed the following question:

"*Under what conditions could it be considered that the concept of 'Closed herds' (where there are controlled and documented conditions of breeding and slaughter), offers the same guarantees as the so called 'BSE-free regions'.*"

In this context, negligible BSE-risk implies that all the animals alive at the moment of certification have never been exposed to any source of infection and have no epidemiological link with TSE cases.

2. Critical factors in the establishment and maintenance of "Closed herds"

Feeding of Meat and Bone Meal (MBM)

It is generally accepted that BSE is mainly, if not entirely, initiated by exposure to contaminated feed where inappropriately prepared MBM is assumed to be the most probable source. MBM should not be fed as long as no guarantee can be provided that it is made solely of animals or materials that presents no risk and are processed appropriately without subsequent (cross-) contamination with TSE infectivity. It is therefore proposed that a negligible BSE-risk herd must be able to prove that no MBM has been fed to any cattle in that herd for at least 8 years. This period is chosen in order to provide a safety margin in comparison to the average incubation period of 5 years. On the level of an individual animal it has to be guaranteed that it never has been exposed to MBM.

Semen and embryos

The SSC considers that the risk of transmission of BSE via semen and embryos is unlikely As a precautionary measure, however, no embryos or semen originating from donor animals, which developed BSE, should have been used in the herd in the previous 8 years. If this happened, all progeny (first generation) should be eliminated.

Live animals

No animal should have been introduced in the preceding 8 years into the 'closed herd' unless sourced from a herd with an equivalent status or from a country or region classified as "negligible till zero BSE-risk".

Vaccines and veterinary medicaments

Vaccines produced in accordance with requirements of the CVMP, are regarded to be safe with respect to the risk of transferring BSE.

Other feed components

Although the risk is regarded to be low, tallow, gelatine, hydrolysed proteins and feed of unknown origin, such as waste food, should not be given.

3. **Information permitting to establish and maintain a "negligible BSE-risk herd"**

a. Disease history

In the previous 8 years no BSE case must have been diagnosed in the herd. Brains from all died or slaughtered bovines from the herd, at an age over 1 year, must be examined in an approved BSE-reference laboratory.

For newly established herds guarantees are needed that the herd is constituted only of animals from a country of negligible to zero BSE-risk or from herds of an equivalent status.

b. Records, surveillance and management

Complete records of births, deaths and all movements of the individual animals for the past eight years are needed.

Veterinary surveillance for recognition of neurological disorders has to be guaranteed.

The herd has to be separated from other domestic species, especially sheep and must have no contact with potentially infected materials.

TSE certification of small ruminant flocks

1. **Definition**

A certified TSE-negligible risk flock is a flock of small ruminants, which gives sufficient guarantees of absence of TSE in the flock after the date that the flock was closed. Guarantee is supported by documented total elimination of all TSE infected and possibly exposed animals and with documented proof of absence of those factors, which could introduce the TSE agent into the flock.

2. **Factors affecting the TSE status of a small ruminant flock**

No TSE may have been diagnosed in the herd since its establishment.

Sheep management and feeding of concentrates possibly containing MBM.

Main differences in small ruminant management and feeding practices are based on its purpose, i.e. meat, wool or dairy, e.g. sheep kept mainly for wool, are most often managed extensively on pasture and not fed concentrates. Hence the risk from feed is expected to be smaller for such sheep as compared to the more intensively managed meat or milk-producing breeds.

Goat management and possible feeding of concentrates containing MBM.

Under certain management regimes, goats are highly at risk if infected MBM is fed.

Feed components other than MBM

Although the risk is regarded to be low, tallow, gelatine, hydrolysed proteins and feed of unknown origin, such as waste food, should not be given.

Feeding and scrapie

It is theoretically possible but not proven that index cases of scrapie could arise from exposure to scrapie-infected MBM. If so, there would be no intra-species barrier for transmission of scrapie via MBM. Initial introduction of scrapie through infected MBM could lead to a smaller or larger epidemic, dependent on the prevailing genotypes of the actual sheep in the region.

Feeding and BSE in small ruminants, should it occur.

Present evidence suggests that index cases of BSE in sheep, if they occurred, would likely be due to exposure to BSE-infected MBM. Current risks would depend on the effective enforcement of MBM bans. Other factors would include cross contamination , bans of specified sheep risk materials (SRM) , rendering parameters, feed processing and scrapie related culling.

Horizontal transmission

Transmission of disease from one animal to another can occur by direct or indirect contact . Potential methods are *via* placenta (proven), milk, faeces or nasal discharges (all unproven). The risk for horizontal spread is the highest when sheep are kept together, for example at lambing time.

Vertical transmission

Infectivity was not found by bioassay of ovine semen from a ram with scrapie, in injected lambs. It remains unclear whether scrapie can be transmitted by embryos.

Environmental and other forms of transmission

Common grazing could constitutes a risk factor, especially around the lambing period but also permanently because of the persistence of infectivity via soil or vectors (hay mites, nematodes, etc). However, the evidence for transmission of natural scrapie from an infected environment is circumstantial.

The evidence for transmission of scrapie via vectors such as hay mites, fly larvae, protozoon parasites and nematodes, is limited. However, this form of transmission cannot be entirely ruled out.

Iatrogenic exposure of scrapie has probably occurred with a louping ill vaccine and a vaccine against *Mycoplasma agalactiae* both prepared from sheep tissues.

Genotype of the flock animals with regard to TSE susceptibility

A flock entirely composed of resistant or semi-resistant genotype(s) is much less likely to have an occurrence of a TSE. If present, infectivity levels in younger animals are likely to be lower as compared to animals of a susceptible genotype. However, according to current knowledge, genotypic resistance will not [yet] provide a 100% full proof of not being a potential carrier of infectivity.

Culling strategies applied to eradicate or control TSE in a flock

Ideally, all animals exposed to the same source of infection as the index case should be culled. Therefore, a culling strategy for small ruminants should cover whole flocks, i.e. where the index case was found and flocks that were in contact with that flock . An exemption could be made for animals of an ARR/ARR genotype.

3. **Information needed to establish and maintain status of a "Small ruminant flock certified as of TSE-negligible risk"**

a. Records

- showing that no clinical cases occurred in the flock.
- indicating that there was a negligible risk that TSE cases were present and that no infectivity was introduced during a given period.
- showing that each animal has been identified and monitored beyond doubt.
- guaranteeing, for newly established flocks, that the flock is constituted only from animals from a country of negligible to zero TSE-risk or from flocks of an equivalent status.

b. Surveillance data

Veterinary surveillance of the flock should be of such level that it is guaranteed that all cases of neurological disorders, for which TSE cannot be

excluded, are immediately recognised. Rapid TSE testing would significantly increase the trustworthiness of a certification.

c. Management

Contact with other flocks is strictly limited to (i) exchanges via artificial insemination, (ii) exchanges between certified flocks and (iii) introduction of ARR/ARR rams for breeding and reproduction.

4. **Elements of an accreditation scheme for maintaining a provisional certificate of representing a negligible TSE risk**

To maintain a certificate of "TSE negligible risk flock", the flock must be kept closed and a number of conditions must be fulfilled:

- marking of all animals
- availability of reliable records
- management practices showing that the risk of introduction of infectivity was/is reduced to a negligible level
- testing of brains from all that have died and from a statistically appropriate number of small ruminants (> 6 months) from the flock slaughtered.

Scenarios for certifications are described in the SSC opinion of 4-5 April 2002 on safe sourcing of small ruminant materials.

Relevant SSC opinions (see annex II): **32,36.**

PREVENTING RECYCLING OF INFECTIVITY: FEED-BANS AND REMOVAL OF RISK MATERIALS

By B. Urlings

The broader issue of recycling animal by-products as feed

The main part of animal by-products is by-products originating from healthy[26] slaughtered animals. An average of 30% of the slaughtered weight composes the volume of slaughter by-products not intended for human consumption. Large volumes of these by-products are processed into highly nutritious animal feed constituents. These processed feed constituents represent very often an ingredient for animal feed production. This can be used in feeding of several species of animals, including petfood and fur animal feed. Recycling of animal by-products as feed should thus be evaluated in a broader context:

- The experience of the emergence of BSE is a vivid illustration of the need to consider precautionary measures before one has absolute proof that a problem has occurred. The possibility of emerging of viruses and other biological agents with unusual characteristics would similarly needed to be evaluated.
- It is also recognised that TSEs occur in many species and experimental evidence that a particular species can develop infection whatever the route of administration (e.g. the intra-cerebral and intravenous routes), is cause for concern, because as yet we have so little information about the natural occurrence of TSEs in different species. Survival of animals in experimental inoculation studies, even for life time, does not provide proof of absolute resistance to infection. Nevertheless a very slow development of disease suggests that the multiplication of the agent is only limited and that the reproduction ratio (R_0) of the disease in a population could be very low, resulting in a fade out of the disease. However a long persistence of a pathogen in a population provides good

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26 Healthy animals are defined as animals which have undergone an ante mortem inspection by an official veterinarian where it was determined that the animals were not suffering from a disease which is communicable to man and animals and that they do not show symptoms or are in a general condition such as to indicate that such disease may occur and they show no symptoms of disease or of a disorder of their general conditions which is likely to make their meat unfit for human consumption. (Definition as given in Directive 64/433/EEC, laying down the rules for ante mortem inspection)

possibilities for the agent to adapt on its host and thus challenging the population again.

- It should also be noted that recycling is a means, whereby such unusual infectious agents can accumulate and/or be amplified in a susceptible species without necessarily presentation of disease.
- Recycling might also similarly lead to biomagnification of toxic substances.
- Many infections are totally or partly species-specific, but infectivity may in some cases require to adapt to new host species on passage, as in experimental models of TSEs. In this context the possible emergence and propagation, after several cycles of recycling, of micro-organisms that are resistant to the standard recycling/rendering processes could also be mentioned.
- The supplementary feeding of herbivorous animals with animal proteins derived from the same or from different species has presented new biological challenges to species that originally evolved to cope only with plant proteins.

With regard to TSE risks resulting from recycling animal by-products, the following elements can be summarised from the various SSC opinions which have addressed this issue:

A. So far there exists no scientific evidence of natural occurrence of TSE in farmed pigs, poultry and fish, which may create a basis for an intra-species progression of a TSE infection due to intra-species recycling.

B. Given the limitations of the surveillance in certain areas, and the length of the incubation time in relation to the normal (=economic or commercial) life span of the animals, it can not be excluded that cases occur and if so, an undetected pool of infectivity could be present.

C. It cannot be entirely excluded, on the basis of the available evidence, that TSEs are already present (albeit undetected) in non-ruminant farmed animals. This is in particular so if there is reason to assume that these species have been (and might still be) exposed to BSE-contaminated feed (produced from ruminants).

D. Recycling of animal material, in general, will increase the risk that cases occur or undetected infectivity pools develop, in particular if potentially BSE (TSE) contaminated material is recycled to ruminants or (possibly) susceptible non-ruminants.

E. Intra-species recycling will, due to the absence of a species barrier, increase the risk further.

F. If recycling, and in particular intra-species recycling, of animal material to farmed animals can not be avoided, all measures that reduce the recycled infectivity would reduce the risk.

G. Measures that reduce the recycled infectivity include[27]:

- exposing the recycled animal material to a treatment by 133°/20'/3b or equivalent conditions,
- excluding those tissues known to carry the highest infectious load (ruminant SRMs[28]),
- excluding[29] fallen stock from the production of feed,
- discontinue feeding pig, poultry or fish potentially contaminated feed a sufficiently long period of time before slaughter in order to reduce the risk of recycling infectivity via the gut-content.

H. It has to be understood that

- the possible measures would not be able to reach a zero risk should infectivity enter the recycling loop, and
- that due to the long incubation time of this type of disease a significant risk would have build up before an incidence becomes visible (as has been seen in the case of BSE in the UK). This proves again the necessity of an effective regional monitoring programme of animal diseases, in order to detect and combat as soon as possible new emerging diseases. Any delay in the control of new emerging, including unknown, diseases poses a risk to human and animal health.

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[27] See also the various opinions of the SSC on the safety of products.

[28] Disease and species dependent, at current only defined for BSE and cattle and cattle, sheep and goats.

[29] For detailed recommendation s see the "Fallen Stock" opinion of the SSC, July 1999.

Ruminant feed-bans and TSE risk reduction

A large number of experiments, abundantly reported in the scientific literature, has shown that cattle and sheep are susceptible to TSEs originating from their own species and that ruminants in general fed with infectious material originating from the same species can be infected with TSEs. Also, experimental evidence shows that BSE can be transmitted to sheep (and goats) via the oral route[30]. Appropriate measures with regard to the avoidance of the intra-species recycling of ruminant by-products will thus play a key role in the prevention of recycling and propagation of TSE infectivity.

If no feed potentially carrying the BSE-agent reaches bovines, the risk of new infections in the cattle population would be negligible. However traces of infectivity may result from cross-contamination of MBM-free cattle feed with MBM-containing pig or poultry feed, e.g. in feed mills that produce both types of feed in the same production lines. Apparently flushing batches that are often used as safeguard against such cross-contamination are not sufficient. Also recipients used for the transport of feed and feed ingredients (boats, containers and trucks) can pose a risk to the transmission of infectivity through cross-contamination. This conclusion from the practical experience is supported by the results of the feeding experiments in UK that have shown that already as little as 0.01 g of infected brain is enough to infect cattle orally.

Removal of ruminant specified risk materials from any feed chain

In BSE infected cattle that approaches the end of the incubation period about 99% of the infectivity is concentrated in the Specified Risk Materials (SRMs). The **Table** hereafter lists the Specified Risk Materials as they are currently defined listed according to GBR categories. Removing these from the feed or food cycle reduces the amount of infectivity by up to two logs. However, small breaches of such a removal mitigates this factor significantly.

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30 See also Section 2 Scope, on other ways of transmission.

Table: **Specified risk materials listed according to GBR categories.**

	Specified risk materials (for animals fit for human consumption)
GBR I	**Cattle:** none **Small ruminants:** none
GBR II and GBR III	Cattle: The skull, including the brain and eyes, tonsils The vertebral column excluding the vertebrae of the tail and the transverse processes of the lumbar and thoracic vertebrae and the wings of the sacrum, but including dorsal root ganglia, and spinal cord of animals above 12 months. Intestine from duodenum to rectum and the mesentery of animals of all ages. **Small ruminants:** the skull including brain and eyes, the tonsils, the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum; The spleen of ovine and caprine animals of all ages.
GBR IV	**Cattle, in addition to the above:** the entire head excluding the tongue, including the brain, eyes, trigeminal ganglia and tonsils; the thymus, the spleen and the spinal cord of animals above 6 months **Small ruminants:** as above for GBR II and III

SRM are not only removed from slaughtered healthy cattle but also from fallen stock or cattle dead at arrival or condemned in ante mortem inspection. If BSE is present in a cattle population the prevalence of infected cattle approaching the end of the BSE incubation period is significantly higher in the sub-population of fallen stock and emergency slaughter than in normal slaughter. Hence excluding fallen stock from the feed chain is effectively reducing the risk of recycling the BSE agent. However, any occasional rendering of fallen stock could clearly pose a high risk.

Relevant SSC opinions (see annex II): **89, 90, 91.**

INACTIVATION BY PROCESSING AND SENSITIVITY OF EXPERIMENTAL ASSAYS

By R.A. Somerville

Despite the application of the principles of safe sourcing of raw materials for the production of animal or human derived products there remain actual or perceived risks of the presence of TSE infectivity. Further risk reduction is often possible through production processes. There are several areas to be considered when evaluating the degree of risk reduction obtained.

The problem

TSE infectivity is notoriously difficult to inactivate. Some TSE infectivity will survive standard autoclaving conditions. The BSE agent strain is particularly thermostable. Under dry heat conditions TSE infectivity shows even greater survival properties, e.g. some infectivity surviving temperatures of 200°C or more. Recent research has started to define these properties and to explore reasons for such high thermostability. It is becoming clear that partial inactivation in some cases with heat or high pH can stabilise residual infectivity. The infectious agent also shows great resistance to chemical inactivation. Although strong protein denaturants such as SDS or Guanidine chloride can be effective, high concentrations and/or long exposure times are required to achieve significant inactivation. Alternatives to inactivation include the use of separation technologies such filtration or phase partition technologies. These can be effective but are sometimes compromised by the heterogeneous properties of TSE infected material.

Methods of spiking

To test an inactivation procedure or a production process it is necessary to prime the input material with a high titre of TSE infectivity. The best source of high titre infected material is from infected brain, using rodent models which achieve and can be assayed to demonstrate high titres relatively quickly and cheaply compared to other possible models. The ideal is to present the infectivity at as high titre as possible but in a form that most likely mimics that of any endogenous infectivity that may enter the process under test. Thus the spike should be from a TSE model that is as similar as possible to the spiked sample with respect to TSE strain, tissue properties and species. However compromise is often required in order to produce an experiment which will produce usable data. Hence

the starting material for the production process (e.g. blood plasma) may bear little resemblance to brain homogenate so the addition of significant amount of brain tissue may compromise the subsequent procedures and the consequent effects on the purification process will have to be assessed. Some prior purification of infectivity may be of value but may also alter its fractionation properties.

Assays

Titration

Titration is the method of choice but is slow and expensive. To determine the amount of infectivity serial dilutions (usually 10 fold) are prepared and injected into groups of recipient animals. Mouse models are preferred. Titrations have to be monitored for prolonged periods to ensure that long incubation period cases are observed. Indeed in some cases infectivity in partially inactivated samples has only been detected after all the animals in control titration of the spike succumbed.

The advantage of full titrations is that they determine the minimum absolute number of infective units present in the sample, although without knowing the efficiency of infection it is not possible to determine the ratio of agent particles to number of infectious units. However it is known that in most intra-species systems the intracerebral route is the most efficient. Other routes of infection are less efficient as are systems in which a species barrier is crossed.

Incubation period assays

Since incubation period is usually inversely correlated to dose, it is possible to estimate the amount of infectivity from a calibrated dose response curve. However the dose response curve is sometimes altered, particularly by some partial inactivation procedures. Accordingly although using incubation period assays can be informative, in experiments where the effect of the test treatment on the dose response curve has not been determined, estimates of titre calculated from an incubation period assay may be compromised.

PrP^{Sc} assays

The protein PrP (sometimes called the prion protein) is found in an abnormal form, denoted PrP^{Sc}, in infected brain. PrP^{Sc} tends to co-purify with infectivity and it is thought that the protein may be a component of the infectious agent. However the relationship between PrP^{Sc} and infectivity is complex and poorly understood. Under some conditions a

substantial proportion of the PrP can be separated from infectivity. In addition some of the abnormal properties of PrP^{Sc} do not necessarily correlate with those of infectivity. Accordingly PrP^{Sc} assays should only be used with great caution as a marker of infectivity since the presence or absence of the protein may not necessarily correlate with infectivity. Moreover the sensitivity of PrP^{Sc} assays is several orders of magnitude less sensitive than infectivity bioassays.

Measuring residual TSE infectivity, and the interpretation of the information obtained

No experiment can demonstrate the absolute destruction or removal of TSE infectivity. What is feasible is the demonstration of a qualitative or quantitative reduction in the amount of infectivity. Such measurements depend on determination of the amount of infectivity used to spike the experiment and compare that with the amount detected after treatment. If no infectivity is detected then it must be assumed that the minimum detectable amount in the assay is the amount remaining. The difference between these two values gives a measure of the clearance factor. In some processes there may be sequential steps that could reduce the amount of infectivity. These steps can be assessed separately but it cannot be assumed that sequential reductions are additive and an overall measurement of the process should also be performed.

Methods of reducing infectivity

Two approaches, destruction or removal are possible. Destruction can include heat denaturation, chemical denaturation with strong detergents or chaotropes, or under more extreme conditions alkaline hydrolysis. Most methods are harsh and may well damage most biological products. Removal by differential centrifugation, precipitation or filtration is also possible. However the efficiency of these methods is often poor. Moreover accumulation of infectivity on filters may cause disposal problems. Infectivity may break through occasionally due to filter failure.

Conclusions

The demonstration of sufficient clearance of TSE infectivity from a product should be only part of risk reduction strategies used. Clearance measures should be based on an assessment of reasonable worst case situations, e.g. where the starting material accidentally came undetected from a highly infected animal. They should remove sufficient infectivity to reduce infectivity in such a reasonable worst case scenario to an

acceptable value. Assessment of the efficacy of the methods should include an assessment of the minimum desirable clearance, the theoretical maximum clearance that the experiment could demonstrate and actual clearance achieved.

Table: Summary overview of current knowledge with regard to TSE infectivity clearance by processing ruminant materials *

Production process	**Infectivity clearance factor**	**Ref: SSC opinion**
Gelatine, alkaline and acid procceses	At least $10^{4,5}$	**46**
Gelatine, heat pressure	At least $10^{6,5}$	
Final production steps of gelatine: filtration**, ion-exchange, rapid UHT sterilisation.	$10^{1.2} - 10^{2.2}$.	
Dicalcium phosphate	At least $10^{3,8}$	**45**
Tri-calcium phosphate	Approx. $10^{4,0}$ (estimate)	
Collagen from hides	No research available, but: hide & skin are not risk materials if contamination is avoided.	**53**
Saturated steam heat/pressure (133°C at 3 bars during 20 minutes) applied on mixture of tissues.	At least $10^{3,0}$	**68, 71**
Tallow, post-sterilisation	Not quantified: >1 and probably $\leq 10^3$.	**52**
Tallow, filtration 0,15% **	$10^{2.8}$ [see also full SSC opinion]	
Tallow, filtration 0,02% **	$10^{3.7}$ [see also full SSC opinion]	
Alkaline hydrolysis at high temperature (150°C) and high pressure.	$10^{3.5} - 10^{4.5}$.	**93**
Tallow derivatives	Total safety assumed under certain conditions.	**44**
Hydrolysates from hair and skin: 1M hydrochloric acid for an hour at temperatures of 65°C or higher leads to almost complete inactivation	Almost complete inactivation, but: hide & skin are not risk materials if contamination is avoided.	**57**

Production process	Infectivity clearance factor	Ref: SSC opinion
Hydrolysates from hair and skin: hydrolysis with 6M hydrochloric acid for six hours at a temperature of 100°C.	Almost complete inactivation, but: hide & skin are not risk materials if contamination is avoided.	57
Hydrolysation of proteins by heat/pressure/time conditions of ≥140°C/≥3.6bar/≥30minutes	At least 10^3	58, 65
Alkaline treatment of hydrolysed proteins at pH≥11, ≥3h at T≥80°C	"further reduces risk" (not quantified)	

* Maximum clearance factors are based on data for reductions achieved from high titre material. The efficiency of clearance is generally reduced when the clearance process is applied to low titre material.

** Inactivation of the agent is considered to be preferable to elimination.

Relevant SSC opinions (see annex II): **44 to 75,103,104.**

DISPOSAL OF RISK ANIMALS (FALLEN RUMINANT STOCK) AND RISK WASTE; A FRAME FOR RISK ASSESSMENTS OF WASTE DISPOSAL PROCEDURES

By J.W. Bridges

Risk animals (fallen ruminant stock) and risk waste and its disposal

At its meeting of 24-25 June 1999 the Scientific Steering Committee adopted a substantial Scientific Opinion on the risks to the public, to animals and to the environment from transmissible biological and chemical agents which may be present in fallen stock and dead animals, including farm animals, fur animals, wild, exotic and zoo animals, laboratory animals, cats and condemned materials as well as on recommendations on how such risks can be minimised. In the light of experience with BSE, this opinion includes consideration of unconventional and as yet unknown agents.

It is known that about fifty per cent of more than 1700 known microbial pathogens can be transmitted by animals to human beings (i.e. They are known to be zoonotic). Human beings may also be exposed to a variety of chemical agents present in food products of animal origin. In some instances biological and/or chemical contaminants have been shown to undergo modification between farm and plate with significant alterations of their risks to health.

Fallen stock dead mammals and non mammals and condemned materials may arise due to a variety of circumstances and can contain one or more of a very wide variety of chemical contaminants and / or biological agents.

Risk to man from dead animals and condemned materials depends on:

- The nature and level of the pathogenic or toxic agent(s) present in the dead animal / fallen stock, which in turn relies on accurate diagnosis and measurement;
- The prospect of intra and interspecies transmission;
- The actual processing / disposal method used;
- The prospects of human exposure as a consequence of the processing / disposal.

The use of Hazard Analysis and Critical Control Points (HACCP) will help to identify critical and other risk conditions. A case by case risk assessment should be conducted whenever possible.

Humans should not be exposed to hazardous agents *via* products recycled from fallen stock and condemned materials. If an animal died or was sacrificed because of a toxic chemical or of a pathogenic biological agent, the fallen stock or suspect condemned material should be disposed of in such a way that any processing into human or animal consumption products is avoided. As it is currently not practicable to expect a surveillance scheme to be applied under all circumstances to guarantee that only fallen stock and condemned material of proper quality are recycled in feed and in view of the potential for post slaughter infection or contamination of low risk material as a consequence of handling, transport and / or storage, the S.S.C. recommended that all material from dead animals where the causes cannot be specified should be considered as condemned.

Regarding the risks from TSEs and unconventional agents, according to current scientific knowledge, inter and intra-species transmission may occur across a range of animal species. The rendering standard of at least 133°C/20'/3 bars cannot, based on currently available data, be considered as totally effective in destroying TSE infectivity possibly present in animal species susceptible to TSE infectivity. Thus, additional protection measures are required to ensure absence of TSE infectivity.

Direct incineration of carcasses and incineration or burning under appropriate controlled conditions of rendered material are economically-feasible technologies for safely disposing of TSE risk materials. A further, but less well evaluated, potentially-suitable method is the treatment of rendered material with lime followed by encapsulation and disposal in a controlled landfill.

Less rigorous requirements, which may include recycling, may be acceptable for TSE-free condemned materials . However, this will depend on the nature and characteristics of the agent involved.

The SSC recognised that in emergency situations it may be necessary, as a short term measure, to seek alternative routes of disposal and it urged that any decision be based on a proper risk assessment to avoid unsafe practices. The competent authorities should carry out such assessments as part of their contingency planning work.

A frame for risk assessments of waste disposal procedures.

The SSC proposed a standard *framework for the assessment of the risk from different options for the safe disposal or use of animal waste which might be contaminated with microbiological agents including TSE.* This provides a structured approach to the assessment of the direct and indirect risks involved in the treatment of materials (potentially contaminated with TSEs or with other pathogens). The framework can be applied to identify suitable processes to be used in a routine situation or in an acute emergency and is intended to assist those preparing a dossier on the assessment of safety of specific processes and/or equipment relating to microbiological agents, including TSE. The proposed framework, however, only covers the assessment of risks directly resulting from the presence of microbiological agents (including TSEs). This framework does not directly address other risks possibly associated with the treatment of animal waste, which may result from chemicals (e.g. hyperchlorite) used in the treatment of the carcass or the material. Moreover, the framework does not address [31] toxic substances possibly present, neither the formation during the treatment of new toxic substances, which may pose a risk to human health and the environment as airborne emissions (for example, dioxins), as effluents or as residues in the treated material (for example, heavy metals).

Safe disposal alternatives to high temperature incineration, in addition to addressing methods for treatment of MBM, should also cover processes for volume reduction of carcasses. It is relevant, in considering safe disposal methods, to identify also the application of any process to pathogens other than prions.

Any decisions on the safety of a particular technology must be based on a sound scientific risk assessment. An essential requisite in utilising any risk assessment framework is to ensure that human health (both health of workers and the general public), animal health and the environment are properly protected. This assurance should be available prior to the widespread adoption of any process for dealing with animal carcasses and derived materials. Although it may be argued that in an emergency situation there is insufficient time for a risk assessment, this practice should be a normal part of contingency planning.

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31 It is understood that the assessment of such risks is covered by other frameworks or scientific opinions and/or by European and/or national legislation for the authorisation of waste treatment, recycling or disposal plants.

According to the legal requirement is defined in Article 4 of the Framework Directive on Waste (96/350/EC), processes and methods, which could harm the environment, should not be used

Typically, the risk assessment of any equipment/facility/ process has two stages:

- Identification of the *generic risk* (i.e. The one intrinsically associated with the specific equipment/ facility/process);
- Identification of *situation specific risks* which may include site sensitivity, effectiveness of the local management systems, etc.

The SSC framework addressed the generic risks only. For a framework to be employed for risk assessment purposes, it must identify each source of human, animal and environmental risk in the risk management chain (See **Figure**). The proposed process as a whole and each of its steps need to be described along with the key operating parameters. In addition, the availability of a flow diagram describing the process as a whole is viewed as most helpful.

The framework comprises the following six components.

1. **Identification of the risk category/categories**

   The categories should preferably be defined according to the 3 levels given in the Animal By-products Regulation (EC) 1774/2002 of the European Parliament and of the Council of 3 October 2002 lays down the health rules concerning animal by-products not intended for human Consumption. These levels are largely based on the aforementioned SSC opinion of 24-25 June 1999 and can be summarised as follows:

   (a) ***Category 1*** comprises of ABP regarded as ***high risk***. This includes amongst others any animals or parts thereof suspected of being infected by a TSE or killed in the context of TSE eradication measures, specified risk material or animals containing such material.

   (b) ***Category 2*** material consists of ABP posing a risk not quite as high as category 1 material but still a high risk. This group includes for example fallen stock and animals killed to eradicate an epizootic disease (other than those under category 1) and products of animal origin containing residues or drugs.

(c) ***Category 3*** material are ABP presenting ***a low risk***. In general, Category 3 ABP are derived from animals or products thereof considered as fit for human consumption but not intended for this use. This category would for example include by-products from the slaughtering process, former foodstuffs of animal origin, fresh fish by-products or catering waste.

2. **Identification and characterisation of risk materials**

Each significant risk material should be identified and an assessment made of the likelihood of human/environmental exposure of 'at risk' groups under:

- normal operating conditions
- emergency/abnormal operating conditions

If significant exposures are deemed possible, an assessment will be needed of the potential risks involved.

3. **Agent risk reduction**

An estimate is required of the degree of the risk reduction (in terms of human health, animal health and the environment) which can be achieved by the process.

This may be based on one or more of the following:

- Direct measurements (preferably, or otherwise:)
- Modelling
- Extrapolation from procedures which were previously proved to be effective in another context.

In each case the evidence to support the estimate must be cited. Where measurements have been made, information on the methodology used should be provided. This would include sensitivity and reliability of the methods used, the nature of samples which have been analysed and evidence that these samples are representative (relevant real samples and the number of tests performed). If surrogates for prion measurement are used, for example analysis of peptide levels, an explanation should be given of their relevance. In any case it is necessary to provide an evaluation of the validity with the uncertainties involved.

4. **Risk containment**

An analysis should be made of the likely effectiveness of the technical measures used to ensure that the risks are contained. It is also necessary to evaluate how these containment measures will operate in the event of the breakdown of the process. Monitoring and surveillance procedures to demonstrate containment need to be specified. If full containment is not achievable, an assessment is required of any potential risk.

5. **Identification of interdependent processes**

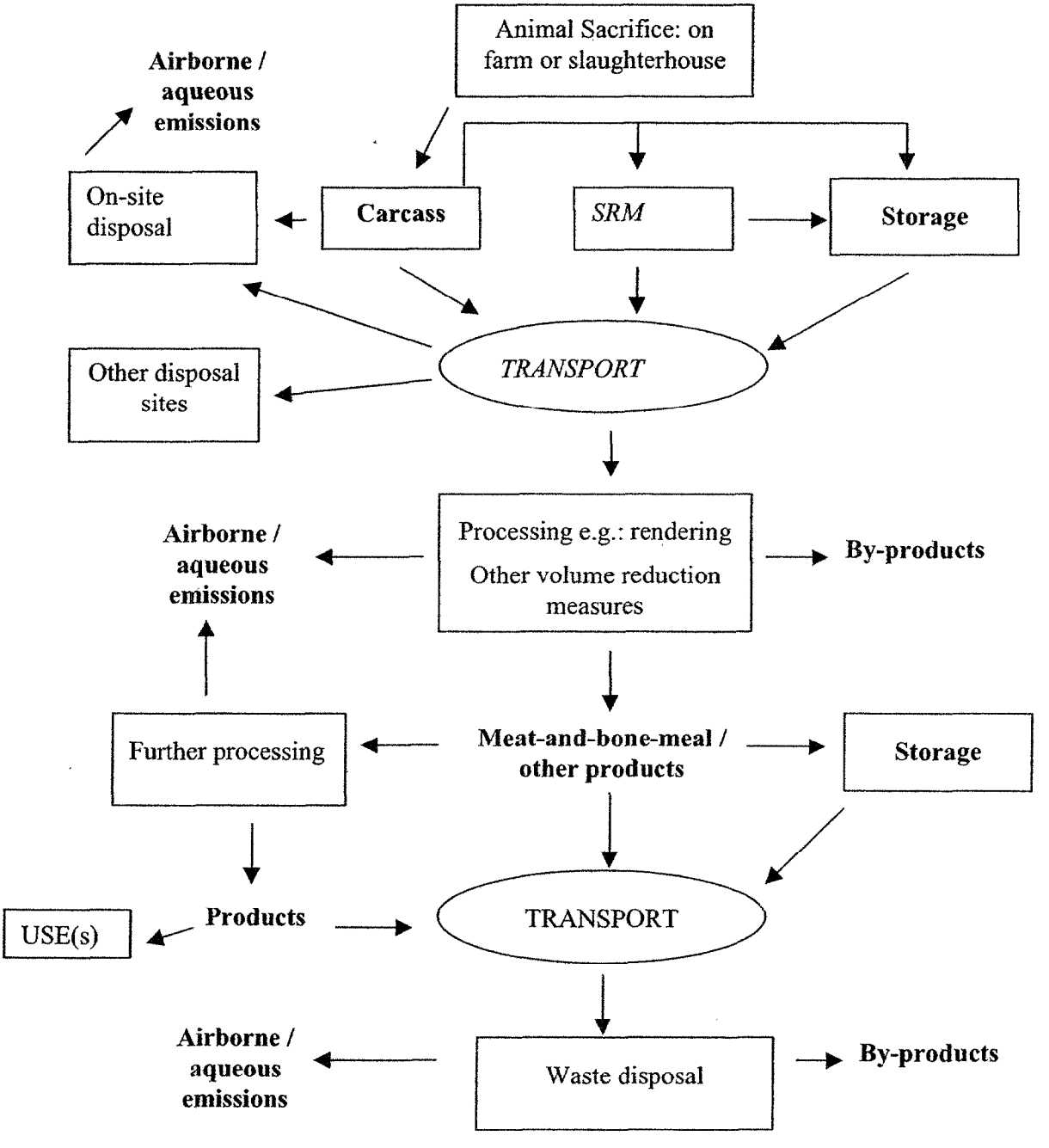
From a risk assessment viewpoint, any process identified to reduce the risk from the agent cannot be considered in isolation from indirect impacts, due to transport, storage and final disposal of the end –products and by-products. These particular aspects need to be evaluated to identify whether an increased indirect risk may occur. For example, risks arising from the increased demand for storage capacity. (See **Figure)**

6. **The intended use of the end-product(s)**

The anticipated uses (e.g. recycling or disposal) of the end-products need to be specified. From the estimated risk reduction (see 2 above), the potential exposure of workers or the public, animal health and/or the environment should be estimated if significant levels of exposure to the product(s) may arise.

Relevant SSC opinions (see annex II): **94, 100, 101, 103, 104.**

Figure: **Risk sources in relation to possible disposal routes for animal derived material, which might be contaminated with a microbiological agent.**



Note: The risk to workers in any of these processes and in handling materials must be assessed fully.

PART II C

SAFETY OF PRODUCTS

SUMMARY OVERVIEW OF SSC OPINIONS ON PRODUCT SAFETY

By M. Vanbelle

The basis of microbiological safety for human consumption of animal-derived products is that the *combination* of several risk reduction strategies will result in a safe end product. This basic principle serves also as the overarching guidance for assuring product safety with regard to the BSE risk. Assessing and reducing the risk of exposure to BSE in ruminant derived products can be divided into five parts:

I. Appropriate sourcing of the animals. [Could the geographical source of animals possibly indicate a BSE risk?]

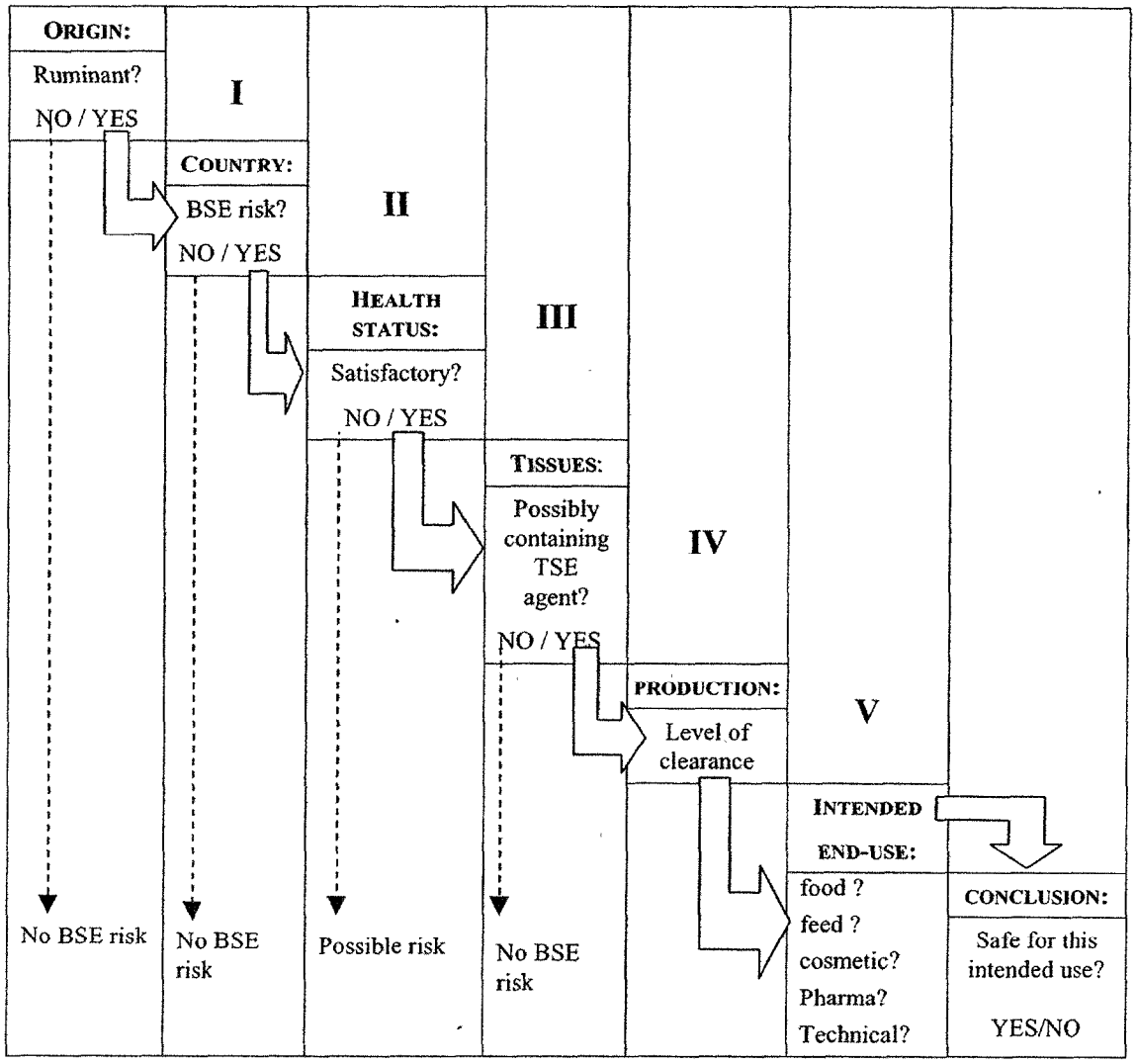
II. Veterinary inspection assuring that the animal is healthy or fit for human consumption. [Does the animal itself possibly poses a BSE risk?]

III. Appropriate sourcing, from a given animal, of the tissues. [Should certain tissues of the animal possibly be excluded for further use? [Are there tissues likely to be infected?]

IV. Appropriate processing of the raw material, resulting in elimination or reduction below significant levels of agents that may still be present after the above steps. [Will the production process remove or destroy TSE infectivity?]

V. Exclusion from certain (human, animal) uses of the product if a doubt remains about the safety of the end product (i.e. certain materials or products should be entirely disposed of or only find applications that exclude human or animal consumption such as certain technical uses.)

The risk of human exposure to BSE infectivity is therefore considered to be reduced to insignificant levels by the *combined* action on all parameters that have a possible impact on the level of BSE infectivity in a cattle-derived product (and in small ruminants products *in case* BSE is detected under natural conditions). This can schematically be presented as follows:



However, sometimes, the application of *all* the above steps is not always required. For example, animals sourced from a country that is proven to be exempt of a certain infectious agent would not need to undergo further risk reduction measures with regard to that agent. On the other hand, certain risk reduction measures may, on their own, result in a very large risk reduction and reduce or eliminate the need for additional measures. An example would be production processes that result in a substantial elimination of an agent.

In the following tables, a summary overview is presented of the safety aspects of a number of ruminant-derived products. In the tables, the following content is given to the notions "specified risk materials".

	Specified risk materials (for animals fit for human consumption)
GBR I	**Cattle:** none **Small ruminants:** none
GBR II and GBR III	**Cattle:** The skull, including the brain and eyes, tonsils, the vertebral column excluding the vertebrae of the tail and the transverse processes of the lumbar and thoracic vertebrae and the wings of the sacrum, but including dorsal root ganglia, and spinal cord of animals above 12 months. Intestine from duodenum to rectum and the mesentery of animals of all ages. **Small ruminants:** the skull including brain and eyes, the tonsils, the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum; The spleen of ovine and caprine animals of all ages.
GBR IV	**Cattle, in addition to the above:** the entire head excluding the tongue, including the brain, eyes, trigeminal ganglia and tonsils; the thymus, the spleen and the spinal cord of animals above 6 months **Small ruminants:** as above

GELATINE FROM BOVINE BONES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants: If: NO						No BSE risk
If: YES	If GBR I:					No BSE risk
	Otherwise	Fit for human consumption? If: NO				Possible risk
		If: YES	Tissues used: long bones Not used: Specified risk materials are removed, including vertebrae, spinal cord, skull, brain; 98% of marrow, lipids, etc. attached to bones are removed by the degreasing step	degreasing, alkaline and acid processes, final filtration and/or sterilisation: Total process clearance: at least $10^{4.5}$. Or: Or: Degreasing, heat/pressure process, final filtration and/or sterilisation: Total process clearance: at least $10^{6.5}$.	Food, feed, cosmetics, pharmaceutical, technical (photographic), etc.	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.
3) Gelatine from bovine bones only represents 23% of total gelatine production in Europe and from this 23%, 50% is used in the photochemical industry

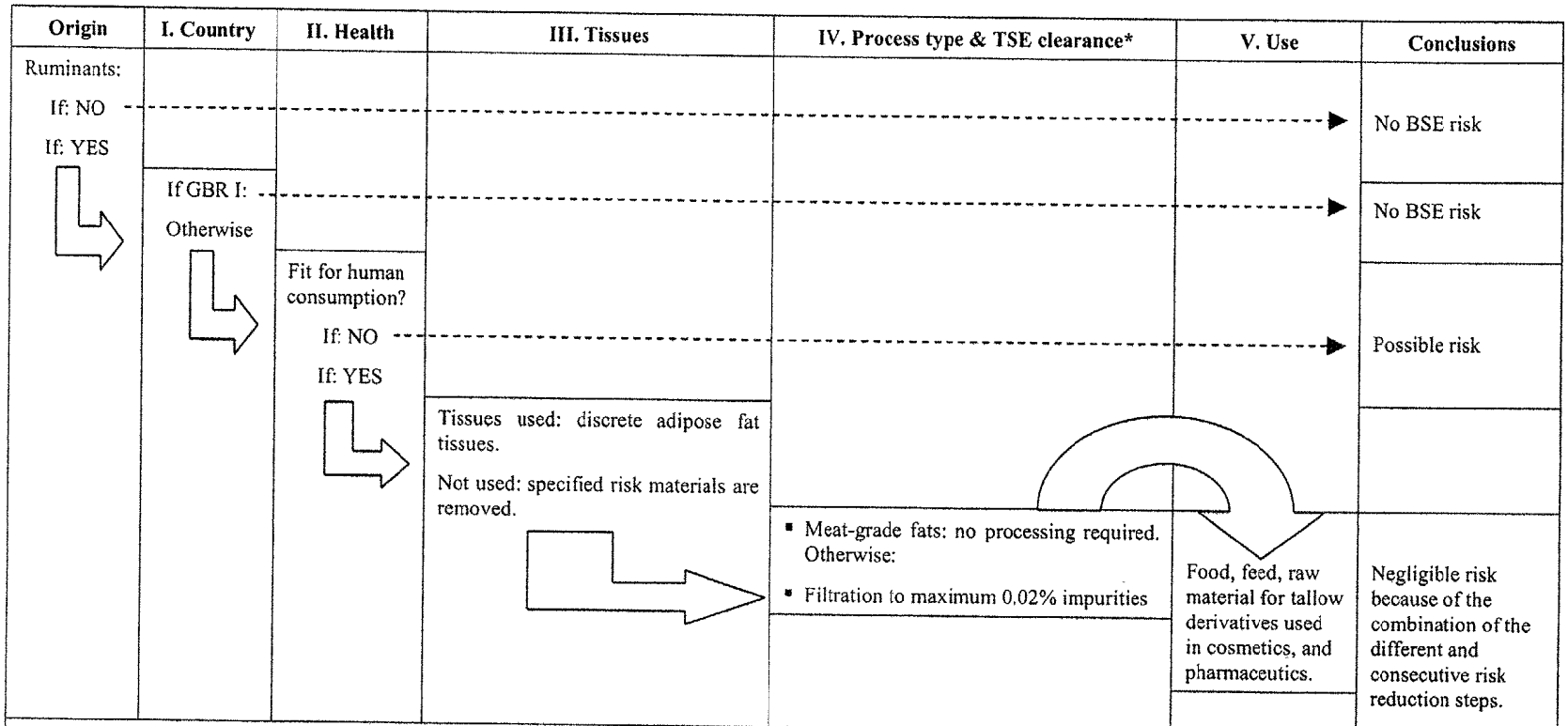
GELATINE FROM BOVINE HIDES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants: If: NO						No BSE risk
If: YES	If GBR I:					No BSE risk
	Otherwise	Fit for human consumption? If: NO				SSC recommends excluding fallen ruminant stock or risk animals from further processing
		If: YES	Raw material used: Bovine Hides (3) Slaughter methods should be such that the risk of contamination with SRM's or potential infected material is prevented. (4)	Process types like as for bones: • Degreasing, alkaline or acid processes; final filtration and/or sterilisation. (Total process clearance: at least $10^{4.5}$) or; • Degreasing, heat/pressure treatment, final filtration and/or sterilisation (Total process clearance at least $10^{6.5}$).	Food, feed, cosmetics, pharmaceutical, technical (photographic) etc	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.
3) Should sheep hides be used and should BSE be found in sheep under natural conditions, an additional risk assessment would be needed for sheep hides with regard to peripheral nerves in subcutaneous layers of the hide.
4) Exclusion of animals that initially passed ante mortem but later tested positive with BSE will further reduce the risk.

TALLOW FROM DISCRETE ADIPOSE TISSUES: RISK ASSESSMENT



Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.

TALLOW FROM MIXTURES OF TISSUES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants:						
If: NO						No BSE risk
If: YES						
	If GBR I:					No BSE risk
	Otherwise					
		Fit for human consumption?				
		If: NO				Possible risk
		If: YES				
			Tissues used: mixture of tissues from which the fat is melted out.			
			Not used: specified risk materials are removed.	• Pre-sterilisation at 133°/20 minutes and at 3 bars and filtration to maximum 0.15% insoluble impurities	Feed; raw material for derivatives used in cosmetics, and pharmaceutics.	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.

2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.

TALLOW DERIVATIVES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Ruminants:						
If: NO					→	No BSE risk
If: YES						
	If GBR I:				→	No BSE risk
	Otherwise					
		Fit for human consumption?				SSC recommends excluding fallen ruminant stock or risk animals from further processing
		If: NO			→	
		If: YES	Raw material used: food- or feed-grade tallow.			
			Not used: specified risk materials are removed.	Hydrolysis at > 200°C for 2 hours and corresponding pressure, followed by either: ▪ <u>To obtain fatty acid esters</u>: Distillation > 200°C. The distilled fatty acids undergo esterification > 200°C with alcohols, followed by a purification to remove (insoluble) impurities; or: ▪ <u>To obtain glycerides</u>: distillation at 140°C. The distilled glycerine undergoes esterification > 200°C with organic acids, followed by a purification to remove (insoluble) impurities.	Food, feed, raw material for tallow derivatives used in cosmetics, and pharmaceutics.	Negligible risk because of the combination of the different and consecutive risk reduction steps, in addition to requirements imposed on tallow used as staring material.

<u>Remarks</u>:

1) It is assumed that the processing is done according to the standards set in the SSC opinions and corresponding EU legislation.
2) "Fit for human consumption" implies a number of conditions assuring that meat from the animal can be safely consumed.

COLLAGEN FROM BOVINE HIDES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Bovines*: If: NO						No BSE risk
If: YES	If GBR I:					No BSE risk
	Otherwise	Fit for human consumption? If: NO				Possible risk; SSC recommends excluding fallen ruminant stock or risk animals from further processing
		If: YES	Raw material used: bovine hides* Slaughter methods should be such that contamination with SRM's or potential infected material is prevented. Exclusion of animals that initially passed ante mortem but later tested positive with BSE will further reduce the risk.	Collagen production generally involves an alkali step followed by an acid extraction step: as for gelatine, the production processes will reduce partially TSE infectivity. No TSE inactivation experiments have so far been carried out with ruminant hides.	▪ Food, feed, cosmetics, pharmaceutical, technical; ▪ Case by case risk assessment for: parental or ophthalmic administration; vaccines; topical application; implantable devices; special grade collagen.	Given the uncertainties/ unknown TSE inactivation capacity of the various collagen processes only an appropriate combination of safe sourcing and end-use will guarantee a reduction of the residual risk to nearly zero

Remark:

* Should sheep hides be used and should BSE be found in sheep under natural conditions, an additional risk assessment would be needed for sheep hides with regard to peripheral nerves in subcutaneous layers of the hide.

HYDROLYSED PROTEINS (PETIDES AND AMINO-ACIDS) FROM BOVINE HIDES: RISK ASSESSMENT

Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Bovines (1)						
If NO:						No BSE risk
If YES	If GBR I:					No BSE risk
	Otherwise:	Healthy animals / animals fit for human consumption?				
		If: NO				Possible BSE risk (contamination)
		If: YES	Tissues used: bovine hides*			
			Not used: any other tissue; also specified risk materials are removed. (2)	For GBR II or GBR III ▪ Brining, liming and intensive washing of hides, followed by a heat treatment at ≥ 140°C, ≥ 3.6 bar (clearance at least 10^3) (3) For GBR IV ▪ In addition to process of GBR II and III and alkaline treatment (pH ≥3.2 and Temp ≥ 80°C) should be applied.	Animal feed and fertiliser	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

1) Should sheep hides be used and should BSE be found in sheep under natural conditions, an additional risk assessment would be needed for sheep hides with regard to peripheral nerves in subcutaneous layers of the hide.

2) Exclusion of hides from animals that successfully passed *ante mortem* inspection, but later tested positive with a *post mortem* test will further reduce risk.

3) A molecular weight of the end product below 10.000 Dalton may be used as an indicator of processing conditions but can not be seen as an absolute guarantee for safety.

AMINO ACIDS FROM HUMAN HAIR HYDROLYSATES: RISK ASSESSMENT

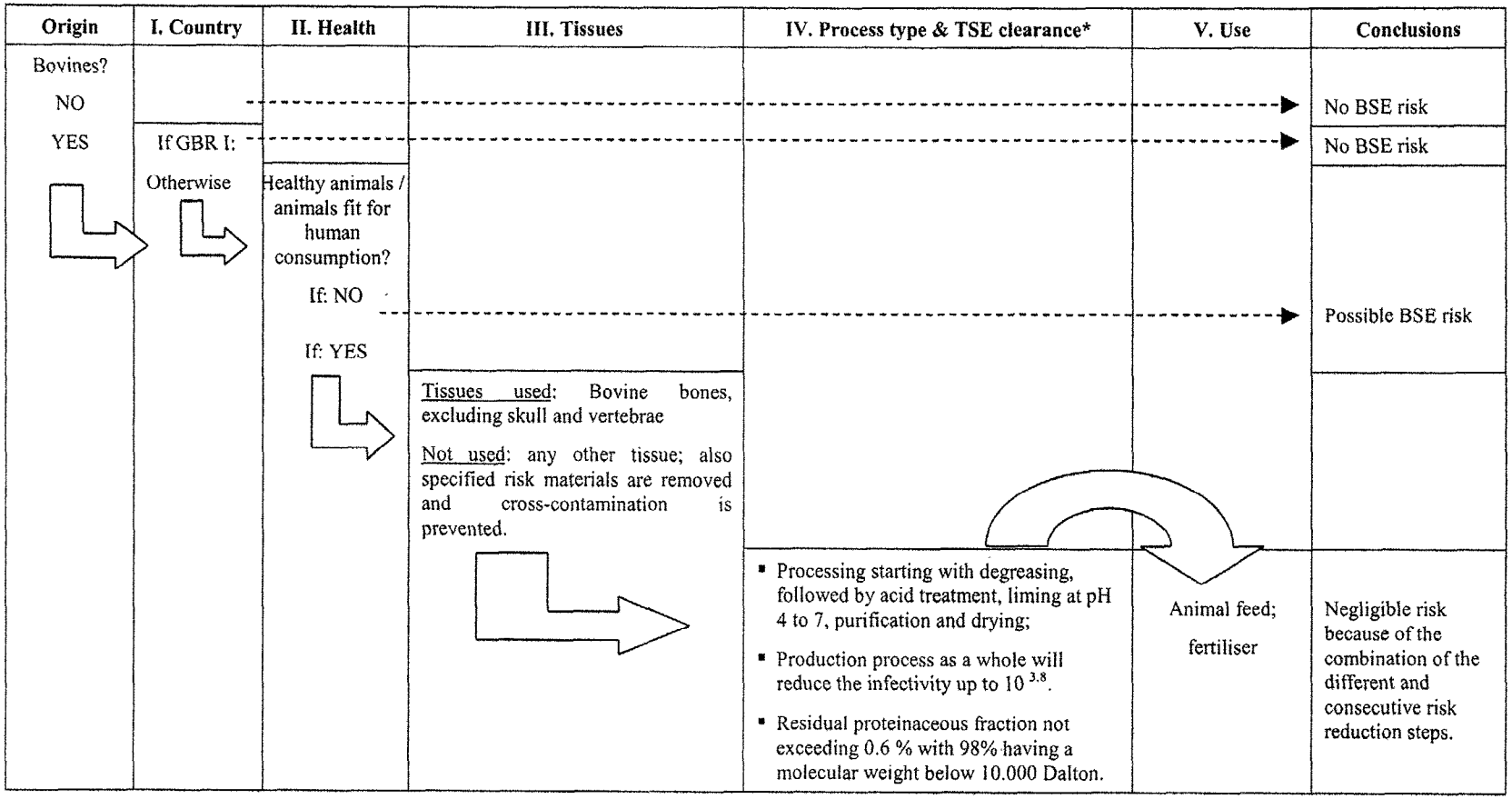
Origin	I. Country	II. Health	III. Tissues	IV. Process type & TSE clearance*	V. Use	Conclusions
Human	GBR I, II, III or IV:	Healthy living individuals?				TSE risk possible?
		If: NO				
		If: YES	Tissues used: Human hair collected by hairdressers and in barbershops, eventually contaminated by skin tissues	▪ Example of a production Process: acid Hydrolysis with 20%, Hydrochloric acid at 100° C, 6 hours;* ▪ Product only containing free amino acids; ▪ Contamination with risk tissues is minimised or excluded; ▪ Crystallisation gives additional purification.	Incorporation into human hair- and skin care products for topical applications **.	Negligible risk because of the combination of the different and consecutive risk reduction steps.

Remarks:

* A molecular weight of the end product below 10.000 Dalton may be used as an indicator of processing conditions but can not be seen as an absolute guarantee for safety.

** No opinion available for other applications / uses.

DICALCIUM PHOSPHATE FROM BOVINE BONES: RISK ASSESSMENT



THE SAFETY OF BOVINE MEAT

By D. Dormont and G. Wells

In the current stage of knowledge, no infectivity has ever been identified in skeletal muscles of naturally TSE-affected animals. Skeletal muscle homogenates from cattle naturally infected with BSE have been assayed in mice. Potential infectivity of skeletal muscles has also been evaluated through the pathogenesis experiment: no infectivity was recorded in several muscles by mouse bioassay, or, to date (Jan. 03), by assay in cattle (intracerebral inoculation), but the latter studies are incomplete (pooled skeletal muscles assays from cattle at different time points in the pathogenesis study are currently 48-76 months post inoculation).

One publication reported the presence of PrP^{Sc} and infectivity in the hindlimb of rodents inoculated with rodent-adapted scrapie strains. Another recent report describes widespread PrP^{Sc} in muscles of hamsters orally infected with a hamster-adapted scrapie strain. PrP^{Sc} was also detected in the tongue of hamsters inoculated by the intracerebral route, with several TSE strains. However, a pilot study was conducted by AFSSA in France, and no PrP^{Sc} was evidenced in either BSE infected mice or BSE-affected cattle.

From these studies it can be hypothesised that very low levels of infectivity may be detectable in skeletal muscles in some experimental models of TSE (rodents). This does not preclude the possibility of the presence of infectivity in skeletal muscle in natural diseases, but meat continues to be considered as not infectious per se.

Relevant SSC opinions (see annex II): **11, 19.**

BSE-RELATED SAFETY ASSESSMENT OF COSMETIC PRODUCTS

By I.R. White and F.H. Kemper

Cosmetic products are normally applied topically (although others may be used on the lips and for oral hygiene purposes). From a human risk exposure point of view, cosmetics are expected to pose less concern than for food or pharmaceutical products in view of the permeability characteristics of human skin including to high molecular weight proteins.

1. Definition

For the purposes of this report, cosmetics are defined as in the Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products:

"A 'cosmetic product' means any substance or preparation intended for placing in contact with the various external parts of the human body (epidermis, hair systems, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or principally to cleaning them or protecting them in order to keep them in good condition, change their appearance, perfume them or correct body odours."

Additionally,

A natural 'ingredient' is understood to mean a substance, a complex of substances or preparations of natural origin, which is used in a cosmetic formulation. (...)"

The above definition also corresponds with the definition used in Council of Europe (2002).

2. The safety assessment of cosmetic products

In general, the assessment of safety-in-use of cosmetic products containing natural ingredients requires integration of two types of data, i.e. those related to toxicity of individual ingredients, and those related to the extent and route(s) of exposure. "Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation" were adopted on 24 October 2000 by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SC-CNFP).

For the assessment of possible residual BSE risks, additional principles and criteria need to be considered. The scientific principles and criteria used since 1997 by the SSC as the bases for its BSE risk assessments are summarised in Part I of this Overview.

A critical factor in the assessment of safety-in-use of a cosmetic ingredient is the extent to which a consumer is likely to be exposed. Exposure to a specific ingredient can be estimated on the basis of:

- *types* of cosmetic containing it;
- *quantity* of the ingredient present in each product;
- *quantity* of each product used by the consumer in each application;
- *duration* and *frequency* of applications of the different products containing the ingredient;
- total *area* of the body exposed to the product in each application, and
- foreseeable misuses which may increase exposure.

Once the exposure has been estimated, the amount likely to enter the body can be estimated on the basis of bio-availability studies.

Other important considerations concerning exposure include the characteristics (e.g. age, atopic status) of the exposed population and other sources of potential exposure to the same ingredient (e.g. by professional/occupational exposure).

3. For assessing the possible residual BSE risk in cosmetic raw materials and ingredients of animal origin, sourced from countries where the BSE risk is *not highly unlikely*, a risk assessment along the scheme presented in the *Summary overview of SSC opinions on Product Safety* by M. Vanbelle (**Part II.C**) is required. If, following this assessment, it appears that a *non-negligible* residual BSE risk may (still) be present in the end product, further evidence supporting the safety of the product and/or a risk assessment is required. Additional key-elements of such evidence may (depending upon the ingredient) include:

   - Whether or not the raw material was sourced from animals fit for human consumption or healthy animals. F*or example:* sourcing of wool for lanolin

from healthy live sheep would exclude the risk of cross-contamination of the hide at slaughter or when processing fallen stock or culled risk animals;

- The degree of purity of an ingredient. F*or example:* careful purification / filtration or crystallisation may exclude the risk that certain substances are contaminated with foreign proteins or long peptides;

- Whether or not cross-contamination was avoided/eliminated. F*or example:* gelatine from bones from countries where a BSE risk is low is safe if an appropriate production process was used, provided the specified risk materials were removed (e.g. skull bones).

4. A detailed discussion of cosmetic ingredients and products would take advantage of a regrouping of the products / substances / ingredients into classes according to the ruminant tissue of origin. To facilitate and scientifically underpin the evaluation of their safety with regard to BSE risks, the products/substances/ingredients can be broadly classified into 3 categories:

   - Products derived from tissues that are proven potential carriers of BSE infectivity and that are in the EU prohibited as "specified risk materials - SRMs". Such products are, for example: brain extract, brain lipids and hydrolysed spinal protein, which should under **no circumstances** be sourced from ruminants from countries where the BSE risk is *not highly unlikely* (GBR I);

   - Products derived from tissues that are proven not to be potential carriers of BSE infectivity. This concerns, for example, wool from live sheep and (probably) hides from healthy animals. Such products are, for example: lanolin and its derivatives, keratin and collagen. These products should in principle be safe, provided cross-contamination with SRMs is avoided.

   - Products derived from various other tissues or mixtures of tissues, where it is not always clear what these tissues are, whether cross-contamination is possible, etc. The safety assessment of such products would require inputs from technicians from the industry on the ruminant tissues that are used for the preparation of certain products/substances/ingredient, on the level of processing and on the level of purification.

5. **Note on products derived from small ruminants (sheep and goats)**

BSE has not been found in domestic flocks of small ruminants nor is there other evidence that BSE is present in small ruminants under field conditions or any indications pointing to an increased likelihood of such being the case. *Scrapie* on the other hand has been recognised for more than 200 years but has not been recognised as contributing to the epidemiology of human TSEs. Therefore sourcing raw materials from small ruminants for the production of cosmetics or ingredients should not result in a human exposure risk to BSE.

On the other hand, the number of small ruminants investigated for the presence of BSE is relatively small, BSE has been transmitted experimentally to TSE-susceptible sheep and natural sheep populations have been exposed to the same feed sources as cattle (albeit probably to a lower extent). The SSC therefore, throughout its opinions, considers it justified that sourcing and processing of tissues from sheep should comply with the same criteria as for cattle. The infectivity distribution in sheep that are susceptible to TSEs is, however, different as compared to cattle (see **Part II.A.**). Therefore, should BSE in sheep be found in small ruminant populations under field conditions, the frame hereafter may need revision for products derived from certain sheep tissues that may be infectious in sheep but not in cattle.

Relevant SSC opinions See *Summary overview of SSC opinions on Product Safety* by M. Vanbelle (**Part II.C**)

THE SAFETY OF PHARMACEUTICALS

By Keith H. Jones and Johannes Löwer

Preamble

The SSC opinions relevant to bovine derived materials used in the manufacture of pharmaceuticals, although they are substantial, are not comprehensive. A comprehensive review has been made and is available in the form of a guideline for the manufacture of medicinal products from the EMEA[32]. This guideline is updated on a regular basis in the light of the most recent scientific and technological evolutions, including the most recent SSC advice. The guideline as a consequence includes reference to all of the relevant SSC opinions. Once adopted, it carries the full force of community law, and will be mandatory for the pharmaceutical manufacturing industry.

A partial discussion of the safety of pharmaceuticals based only on the opinions of SSC would therefore be of limited value. This section is therefore limited to a statement of the scientific principles involved in assessing the risk for transmission of BSE as a result of using bovine derived materials in the manufacture of pharmaceuticals, but reference to the comprehensive guideline (EMEA/410/01 Rev. 1) is given:
http://www.emea.eu.int/
http://www.emea.eu.int/index/indexh1.htm
http://www.emea.eu.int/pdfs/vet/regaffair/041001en.pdf

A discussion of the safety of pharmaceuticals with respect to spongiform encephalopathies would not be complete if it would not include the risk posed by human forms of TSEs may they be linked to BSE or not. Therefore, SSC's TSE/BSE ad hoc working party and the Scientific Committee for Medicinal Products and Medical Devices (SCMPMD) analysed in great detail the possibility that Creutzfeldt-Jakob disease (CJD) or its variant (vCJD) might be transmitted by blood, blood products or human organs or tissues.

The safety of pharmaceuticals

The transmission of spongiform encephalopathy by medicinal products has been a matter of concern since before the recent epidemic of BSE. The effectiveness of transmission of spongiform encephalopathy by pharmaceuticals has been clearly demonstrated in veterinary medicine by the transmission of scrapie by looping ill vaccine prepared from

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[32] EMEA: The European Agency for the Evcaluation of Medicial Products.

ovine spleen and brain; and in human medicine by human growth hormone prepared from human cadaveric pituitary glands and by human dura mater preparations.

The potential for transmission of spongiform encephalopathy by pharmaceuticals is substantial because more than 95% of medicinal products used in human and veterinary medicine are manufactured using materials of bovine origin. These include gelatine used for capsules or as a carrier or stabilising agent; tallow and tallow derivatives - particularly stearates used as filling agents; bovine derived wetting agents; bovine serum albumin and calf serum used as stabilising agents and as components of cell culture media in the manufacture of vaccines and other 'biologically derived' medicines; rennin used in the production of lactose; amino acids derived from hair and skin.

The principles which apply to limiting the risk of transmitting TSEs via medicinal products are those already recommended by the SSC for all other areas, namely:
safe sourcing
tissue selection
rigorous processing
limiting use to specified applications.

These principles and SSC opinions based on them have been used to develop recommendations for the manufacture of bovine derived materials used in the manufacture of medicinal products. Accordingly, specific SSC opinions have been delivered on the manufacture of gelatine, tallow and tallow derivatives, rennet and amino acids. These recommendations propose conditions and precautions to be used during the manufacture of each of these materials of bovine origin so that they can be further used in the manufacture of medicinal products.

A detailed analysis of experimental and epidemiological data lead to the conclusion that classical forms of CJD, although, on several occasions, transmitted by pharmaceuticals derived from tissues of the central nervous system or adjacent tissues, are not transmitted by blood components or blood products. However, as the experience with vCJD which differs in tissue distribution from CJD is limited the advice was given to follow a cautionary approach with respect to the possibility of vCJD transmission by blood or blood products. Quite a number of possible measures following the precautionary principle were discussed in a series of Opinions. They include the exclusion of plasma from donors who lived in countries with a high risk for vCJD or the introduction of general leucoreduction.

Risk/benefit considerations

Special considerations apply to the risk assessment of medicinal products as a result of the benefit that should be derived from their use and whether that benefit relates solely to the individual exposed or more widely to the population.

Medicines are most frequently administered for the benefit of individuals suffering from the effects of disease where they might be expected to bring direct benefit to the individual exposed to the risk. They are also used for the prevention of disease in otherwise healthy subjects, often of young age, where an important objective might be the achievement of a population benefit as well as protection of the individual. Under the latter circumstances a much greater benefit or lower degree of risk is required.

These considerations may make additional or lesser degrees of risk acceptable or contribute to a more rigorous approach to the risk assessment of medicinal products indicated for prophylactic use. Other factors such as route of administration, dose, age, presence or absence of concurrent disease, frequency and duration of treatment are also important considerations. For these reasons the risk assessment process for pharmaceuticals must be made on a product by product, or case by case basis. Furthermore they are often based on rapidly evolving science and need to be updated regularly as the knowledge base moves forward.

Relevant opinions: See *Summary overview of SSC opinions on Product Safety* by M. Vanbelle **(Part II.C); 78**

Relevant opinions of the Scientific Committee for Medicinal Products and Medical Devices:

Opinion and report on the equivalency of alternative products to intestines of animal origin for use as surgical sutures, adopted on 16 September 1998

Opinion on the risk quantification for CJD transmission via substances of human origin, adopted on 21 October 1998

Opinion on the Safety of Hides and Skins, adopted on 24 March 1999

Opinion on the Policy Regarding the Use of Blood and Blood Products adopted by Written Procedure on 24 March 1999

Opinion on update of the opinion on the Risk Quantification for CJD Transmission via Substances of Human Origin, adopted on 16 February 2000

Opinion on the safety of Human-Derived Products with regard TSEs adopted on 18 January 2002

THE SAFETY OF RUMINANT BLOOD

By H.Budka

There is concern that animal TSEs might be spread by blood that has been used as food or feed, as fertiliser on pasture, or through specific blood components or blood based products that are still permitted to enter the market, including medicinal products and biologicals. While normally TSE risks are controlled by a combination of factors including production processes that are likely to contribute to some reduction of prion infectivity, the situation here is different: usually ruminant blood is used without any treatment that is able to decontaminate prions. Thus only sourcing, type of use and potential for contamination remain the key factors to control for TSE safety of ruminant blood.

Experimental studies on TSE infectivity in blood and its components

The majority of bioassays on infectivity in blood have been carried out in animals with clinically overt TSE. In consequence there is substantial ignorance about the early pathogenetic involvement of blood, especially in naturally occurring diseases. In BSE, transmission has not been achieved in natural disease, but blood has been shown to be infectious in experimental BSE in genotypically susceptible sheep and in sheep with naturally occurring scrapie after transfusion of large blood volumes. In experimental scrapie, blood components obtained during both the pre-clinical and clinical stages of disease from rodents, have revealed the presence of the infectious agent. In sum, while epidemiological evidence has so far failed to identify any blood-related cases of TSEs, data from both experimentally induced and natural TSEs suggest that blood has at least the potential to transmit disease.

Use and Sourcing

Slaughtered cattle, sheep, goats and deer could supply blood for food, feed and other purposes. All these species are susceptible to TSEs both naturally and experimentally. BSE as a natural disease has only been reported in cattle. The possibility of BSE being in sheep and goats cannot be excluded. No validated tests exist to detect TSE in live cattle, sheep, goats or deer. Close surveillance for the disease and effective ante mortem clinical inspection of all slaughter animals therefore remain essential.

Risk Assessment

Apart from the potential risk that ruminant blood might contain very low levels of endogenous infectivity, the question of contamination of blood from external sources must be addressed, in particular the possibility of brain tissue contamination at slaughter. Thus the most important aspect of risk relates here to such contamination.

1. the amount of brain material actually entering the bloodstream following the use of invasive stunning devices. Neither its volume range nor the range of particle size is known. Likewise, no quantitative estimates are available on contamination of blood with SRM materials during the slaughtering process other than by stunning the animals.
2. dilution of CNS material resulting from the emboli, and
3. the efficacy of the various processing steps in respect to inactivating the BSE agent.

There is little doubt that under certain circumstances, humans or animals could be exposed to the BSE agent by consuming blood products.

The SSC proposes a general approach for the risk assessment for blood within a given area, which basically involves 3 aspects:

Slaughterhouse

At the level of each slaughterhouse, the following risk factors should be in particular evaluated in respect to:

1. number, species and age of slaughtered animals;
2. number of potentially infected cows being killed and their brain material entering the bloodstream related to the stunning method used (non-penetrative vs. captive bolt, pneumatic devices, pithing);
3. the average amount of blood collected per animal;
4. the dilution by pooling blood from several animals;
5. the amount of such collected blood going to the industry to be processed for human or animal consumption.

The TSE risk derives in particular from the following factors:

- The highest risk of producing CNS emboli follows captive bolt stunning with compressed air into the cranial cavity.
- Cartridge operated captive bolt stunning followed by pithing presents the next highest risk.

There is insufficient knowledge to advise on the degree of risk from the use of penetrative cartridge-operated stuns without pithing, free bullets or non-penetrative guns.

There are no published papers on the effect of various stunning methods on sheep and goats and in regard to the generation of CNS emboli.

More information is required on the possible dissemination of CNS emboli into the systemic circulation.

TSE risks may exist as a result of the source of animals for slaughter.

TSE risks may occur independently of the stunning procedure, as result of TSE-infected material from SRM entering the blood after exit from the body.

Geographical BSE risk and surveillance

For the geographical BSE risk and surveillance reference is made to the GBR opinions adopted by the SSC.

The use of blood

At present, blood collected hygienically in licensed EU abattoirs can be used for food, feed and a variety of other purposes with or without any form of processing, including cosmetics, pharmaceuticals and technical use as fertiliser. For example, it is permissible to incorporate fresh untreated plasma into the materials used for the production of sausages, and it can be spread on land as a fertiliser. However, there could be a risk of the occasional presence of low levels of TSE infectivity in blood collected in abattoirs. Levels of infectivity that might represent a risk to animal or human health are not known. Control measures and/or decontamination standards thus might need to be developed to potentially TSE-infected blood collected in abattoirs.

Conclusions

For ruminant blood, the best approach to protect public health at present seems to assume that it could contain low levels of infectivity. However, even if this is true, it becomes almost irrelevant compared with the level of contamination that could occur as a result of the methods of stunning used in abattoirs. These procedures have been recognised to release particles of brain tissue (potentially containing high titres of TSE infectivity) into the bloodstream. The frequency at which this occurs appears to increase with the severity of the stunning process, and this is an area requiring further research. There are also opportunities for the contamination of pooled blood as a consequence of the release of brain tissue from the hole left by stunning, or with spinal cord during its removal (if a production-line process is not used). Nevertheless, given the low frequency at which

apparently healthy animals testing negative in a rapid post-mortem TSE test would have TSE infectivity in the CNS at the time of slaughter, it is considered that the overall potential level of infectivity in pooled blood will be low.

A summary of the SSC opinion on the safety of ruminant blood is given in the **Table** at the end of this contribution.

Recommendations

The SSC recommends that its opinion on the safety of ruminant blood is considered in conjunction with its opinions on "Fallen stock" (June 1999), "Intra-species Recycling" (June 1999), and "Stunning methods and BSE risks" (January 2002).

Consideration should further be given to avoid methods of captive bolt stunning with compressed air or followed by pithing ruminant food animals that increase the risk of CNS material entering the blood stream at slaughter wherever there is a significant risk from TSE[33]. In addition, sourcing from young[34] animals would further reduce the risk.

Improved methods for reducing the risk of cross contaminating blood with CNS or other SRM post-collection need to be developed or put in place where necessary. Brain spilling from the bullet hole into the blood tank should be prevented; surveys should check the absence of brain material in the blood tanks.

Where an element of risk is perceived, this may be reduced or eliminated by (a combination of) various strategies, as follows:

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33 Changing from pneumatic stunning or pithing, to stunning methods that avoid severe brain damage could go along with an increased risk of physical injury to slaughtermen (particularly during shackling and bleeding out) if the new methods or building facilities are not properly designed.

34 First infectivity in CNS of cattle is detected in most cases in the last quarter of the incubation period. Defining young animals could be done on the basis of the probability of occurrence of BSE according to the age. (See for example the annexes 3 and 4 of the Opinion of 28-29 October 1999 of the Scientific Steering Committee on the *Scientific Grounds of the Advice of 30 September 1999 of the French Food Safety Agency (the Agence Française de Sécurité Sanitaire des Aliments, AFSSA), to the French Government on the Draft Decree amending the Decree of 28 October 1998 establishing specific measures applicable to certain products of bovine origin exported from the United Kingdom.*

Source bovine blood from BSE-free areas or closed herds or other schemes that reduce to a minimum the probability of an animal being infected;

Subject the product to a "133°C/3bar/20'" autoclaving or equivalent validated process.

Pharmaceuticals including vaccines are regulated products, and the use of bovine derived blood products in their manufacture is controlled on a case by case basis. The basic principles reviewed here should of course be respected.

Relevant SSC opinions (see annex II): **13,20, 21, 26, 28.**

Table: Summary of the SSC opinion on the safety of ruminant blood.

GBR*	HUMAN FOOD	ANIMAL FEED	COSMETIC	PHARMACEUTICAL	TECHNICAL (FERTILISER)
I	No risk with regard to BSE				
II III IV	As for meat: animals fit for human consumption, appropriate slaughtering process (without pithing/contamination by SRMs) and blood collection technique, avoidance of cross-contamination, etc.	As for food and: Avoidance of intra-species (ruminant) recycling	As for food	For oral and limited topical administration: as for food. Otherwise: a case-by-case risk assessment for: – parenteral and ophthalmic administration; – topical administration to large skin areas of open wounds; – vaccines; – implantable devices	As for feed, i.e. blood recuperated from animals at risk or part of an eradication programme, should not be disposed of as a fertiliser.

* Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a region or country: Unlikely but not excluded (GBR II); Likely but not confirmed or confirmed, at a lower level (GBR III); Confirmed, at a higher level (GBR IV)

Note: The situation with blood differs from all other materials as processing normally does not reduce any infectivity. The SSC thus stated that "*Sourcing from young animals would further reduce the risk*" and "*Where an element of risk is perceived (this would apply to GBR II-IV), this may be reduced or eliminated by:*

- *sourcing from BSE-free areas or closed herds or other schemes....; and:*
- *subject to 133°/3 bar/20'".*

THE SAFETY OF NATURAL CASINGS DERIVED FROM THE SMALL INTESTINE OF SMALL RUMINANTS (SHEEP AND GOATS)

By R. Bradley

Definitions

The term casing refers to the envelope enclosing an animal product, principally containing meat, offals (like liver) or blood for human consumption, the whole being termed a sausage. This report refers only to the TSE risks in natural casings derived from the small intestine (duodenum to ileo-caecal junction inclusive) of small ruminants (sheep and goats), only to the casing and not to the contents. It cannot be excluded that small local enterprises harvest large intestines for local use.

Intestines used to produce natural casings are only sourced from animals destined for human consumption, slaughtered in licensed abattoirs following official *ante* and *post mortem* examination and passed fit for human consumption. The whole process of slaughter and subsequent procedures in the abattoir are in principle subject to official control.

General statements on the report

Consultation with members of the Scientific Working Group of the International Natural Casing Associations resulted in the following comments:

Desliming is the most important factor that influences the quality of natural casings with respect to marketing aspects and can be achieved by machine processing or manual processing. Machine processing is achieved by passing runners through a series of cleaning machines and tanks of hot water during which both the inner and outer layers of the small intestine are removed. (The inner layer, or mucosa, is that part which is believed to contain most of the infectivity in an infected intestine). Finally, the casings are passed through a finishing machine. Quality control is continual, and additional checks are made, such as measuring the gauge of the casings and making sure there are no holes. Any faulty casings are discarded. The runners are collected into hanks of 50, salted and placed in barrels of salt (sodium chloride) for a minimum of 30 days, prior to dispatch.

There are no commercially detectable differences in casings processed by hand or machinery.

It is known that sheep and goats can be experimentally infected with the agent that causes BSE and develop fatal spongiform encephalopathy. However, natural cases of BSE in sheep have not been reported in any country to date. If BSE is found in sheep or goats in the future there is a clear potential risk for humans as BSE is a zoonosis. The agent causing the experimental disease 'BSE in sheep', or more accurately 'scrapie caused by the BSE agent' (because the clinical and pathological features are closely similar to those of scrapie) is biologically indistinguishable from the agent that causes variant Creutzfeldt-Jakob disease of man. Precautionary risk reduction measures have been applied to certain risk sheep tissues (such as the skull, brain, eyes, spinal cord and spleen which are designated specified risk materials (SRM) that must be destroyed) in the EU and some other countries.

Small ruminants can be naturally affected by scrapie, a TSE naturally confined to these species, caused by various strains of scrapie agent (that is biologically and molecularly different from the BSE agent) and which are not regarded as human pathogens. Much of the risk assessment for BSE in sheep is based on knowledge from natural or experimental scrapie in sheep and goats. No formal action is taken against scrapie in regard to public health except that scrapie is a notifiable disease in the EU and animals suspected clinically to have scrapie are prohibited to enter any food or feed chain. Removal of SRM from sheep and goats reduces exposure of man and animals to scrapie (and to BSE if it occurs) even though the driving force for the legislation was the fear of BSE being found in sheep in the future.

Tissue distribution of infectivity in sheep and goats with natural TSE

What the tissue distribution of the BSE agent would be in natural BSE in small ruminants is unknown, because the disease is hypothetical and speculative. However, it is more likely to be similar to the distribution of scrapie agent in natural scrapie in sheep and goats than to natural BSE in cattle. That is, it would have a wide distribution in lymphoreticular tissues and nervous tissues. The brain, eye, spinal cord, associated ganglia, intestine, lymph nodes, and possibly other tissues come into consideration. Like scrapie, genetic resistance can occur and immunohistochemical detection of PrP can determine the precise sites of accumulation of prion protein in infected organs. Some studies have identified PrP not only in the gut-associated lymphoid tissue (GALT) but also in the enteric nerve plexuses (Auerbach's and Meissner's plexus). Thus any TSE-risk reduction resulting from the process of making a natural casing will be related to the completeness of the removal of the GALT and the two nerve plexuses,

Infectivity titres in intestinal and other tissues

An important missing component at the time of writing is the absence of data on the amount (titre) of infectivity in any infected tissues of experimentally BSE-infected sheep or goats. This is because there are no reports of infectivity titrations, including for the intestine. It is assumed that any titres that are present may be closely similar to those published for goats, in the clinical phase of scrapie and Suffolk and other breeds of sheep in the pre-clinical and clinical phase of natural disease. Unfortunately even these detailed studies did not investigate the titre of infectivity in parts of the small intestine other than the distal ileum which is rich in lymphatic tissue in the form of Peyer's patches. Infectivity in the distal ileum was consistent from at least 10 months of age when detectable infectivity was also present in spleen and lymph nodes. In some breeds, individual sheep with natural scrapie, confirmed by microscopic examination of the brain, had no detectable infectivity at all in the ileum, and in one case in a Montadale sheep, none in any tissue, except the CNS.

When significant levels of infectivity were found in the ileum they were of the same order of magnitude as in lymph nodes from a wide range of body sites and in spleen and tonsil. Thus it would seem logical that if a TSE risk were perceived for the intestine, then lymph nodes also would present a risk. Lymph nodes are present in some cuts of bone-in meat. The highest risk part of the intestine is presumed to be the ileum since it is the part with a consistently high level of GALT and usually has (scrapie) infectivity if other lymphoreticular tissues are infected.

In regard to natural casings, as distinct from intestine, if the presumed infected lymphatic tissue is removed before sale to the public, the TSE risk in the lymphatic tissue would be removed along with it, disregarding for the moment risks from cross-contamination. Partial removal would result in a risk reduction, though not elimination of infectivity in the GALT. Even if GALT were completely removed any infectivity in Meissner's plexus would remain as this is within the sub-mucosa that forms the casing. It therefore becomes important to determine:

If infectivity (as distinct from PrP) is present in the sub-mucosal nerve plexus

How much this contributes to the infectivity of the intestine as a whole.

It is noted that random-bred, female Swiss mice were used for the original bioassays of scrapie infectivity, which are likely to underestimate the real infectivity by some unknown factor because of the species barrier between sheep and mice. Thus the 'real' titres determined by i/c inoculation of sheep of the same susceptible *PrP* genotype may be higher than those reported. Therefore, any estimate of the reduction in risk by processing any material from infected sheep, including natural casings, might be

correspondingly larger than currently envisaged (for example, 80% reduction of 6 logs of infectivity is more efficient than 80% reduction of 2 logs of infectivity).

Parts of the intestine in which TSE infectivity may reside

Collectively, research studies show that tissue exists in the intestine of sheep that is able to harbour, and possibly replicate, TSE agents including BSE. These studies show that tissue exists in the intestine of sheep that is able to harbour/replicate TSE, including BSE, infectivity. These tissues are GALT, nerve cells and glia within the two nerve plexuses of the gut. In regard to GALT, FDC probably contribute the highest amount of PrP within the Peyer's patch. Intestinal dendritic cells and tingible body macrophages (both of which are mobile cells) and M cells probably contribute less.

The age of source animals and age at which intestine is infected

Casings are estimated to be collected from about 85% of slaughtered sheep. The age range might be estimated to be as follows in the UK: < 6 months 8.6 millions, (only in this group could infectivity (if present) be assumed to be at a low titre or absent) 6-12 months 5.5 millions and > 12 months, 1.9 millions.

If sheep were infected with BSE *via* feed in most instances this exposure is more likely to occur later (e.g. after weaning) than if infection came from other sheep (including the dam) or the environment when exposure would likely be higher immediately following birth than later. Nevertheless, evidence from research in experimentally challenged sheep shows that prion protein can be detected in the Peyer's patches of the intestine in some *PrP* genotypes of sheep at a relatively young age (5 months) but not in the enteric nervous system until 10 months. Guarantees cannot be given of freedom from infectivity by age.

Part of the small intestine used for natural casings

For some years, the European natural casings industry has been advised to, and in practice does, remove the whole of the ileum and a short part of terminal jejunum before preparing the intestine for processing into casings. This is a HACCP procedure in the European industry.

Risks from cross contamination in the abattoir

Currently electrical stunning, which is the most common method used for stunning small ruminants, is regarded as presenting a negligible risk of *embolic spread of brain tissue* to the blood stream. In some abattoirs (particularly those with a low throughput), may stun sheep by methods that penetrate the skull and damage the brain. A cartridge operated captive bolt pistol can cause brain emboli to enter the venous system in sheep and is still

permitted in the EU but more research has been advised to confirm the observation. Other risk methods of stunning food animals are banned in the EU.

A wide range of tissues (including the current SRM) could carry BSE infectivity and in theory might be a *source of infection for cross-contamination of intestine*. However, in practice these theoretical risks can be eliminated by careful application of meat hygiene rules.

The risks linked to *meat-and-bone-meal or BSE-contaminated bovine fat feeding* can now be considered as negligible.

Factors to be taken into account when making an assessment of the risk to the consumer from natural casings and bone-in meat

The absolute amount of infectivity remaining in a prepared casing is the important criterion in determining the TSE-risk for the consumer. In this regard it is important to also take account of the dose of infectivity that a consumer might consume at one meal. Casings are only eaten as an envelope of sausages rather than as a commodity on its own. Casings therefore contribute a relatively small amount by weight to a meal of sausages. Thus the dose of infectivity that might be consumed (if residual infectivity was present) will be calculated as a product of the weight of the casing multiplied by the absolute residual infectivity titre per unit mass of the casing. This contrasts with the higher theoretical TSE-risk in bone-in meat from the same infected animal (because of its content of possibly infected lymph nodes, peripheral nerves and bone marrow).

The risk analysis

The BSE agent has not been isolated from any sheep or goat with a scrapie-like disease or indeed any sheep under natural conditions. At the present time (2003) the hazard is therefore a hypothetical one.

From all the above data and if BSE were to occur in sheep or goats the following can be stated:

Source

Intestines harvested for casing manufacture come only from animals passed fit for human consumption in a licensed abattoir.

Small intestines from sheep could harbour the BSE agent. In regard to age, no exclusion can be made but small intestines from animals under six months of age are likely to present, on average, a lower risk than intestines from older animals. The *PrP* genotype could have a greater bearing on the infectivity at different ages of exposed sheep. The

degree of infectivity in the intestine at any particular age is likely to be very similar to that in the spleen (SRM) an in lymph nodes (not SRM) and form part of the sheep sold to the public.

Within the intestine, the ileum (notably the distal ileum) is likely to have a clearly detectable level of infectivity. Infectivity in other parts cannot be excluded.

Process

In the EU, Switzerland and in some other countries no part of the large intestine or the whole ileum and short piece of terminal jejunum of inconsistent length is used for casing manufacture and trade. Any TSE risk in these tissues is removed. The risk of cross contamination of the remaining part of intestine that is used (duodenum and most of the jejunum) by SRM or other BSE-infected material is negligible provided the EC meat hygiene and other regulations are complied with and enforced.

The cleaning operation removes half of any infectivity present in the enteric nervous tissue. In addition, removal of the mucosa and Peyer's patches is efficient but could not be guaranteed to be complete in all parts of all casings. In some parts it will be perfectly removed and in others not. Overall on average over 80% of this tissue is removed and it could be almost 100%. There are no data on starting or finishing titres so it is not possible to be precise about the amount of infectivity removed but it is estimated to be at least 2 logs and possibly over 3 logs. It is noted that no risk reduction is achieved on consumer sales of bone-in meat like a leg of lamb from the same animal. Some structures in the leg could contain infectivity.

Use

Natural sheep and goat casings have only one use, namely as an outer envelope for sausages. No TSE risk reduction occurs during the filling process. When sausages are cooked the outer surface (the casing) reaches a temperature of about 175°C for some minutes. This may have some inactivating effect on TSE agents present in the casing but it could not be guaranteed to sterilise the casing.

In regard to human consumption the contents of a sausage contributes by far the greatest mass to a sausage meal with the casing contributing only a few grams (< 4.0g).

Conclusion

A significant BSE risk-reduction is achieved by the EU and Swiss sheep and goat natural casings industry. This is secured by eliminating at source those parts of the intestine with the highest risk and by removal of most of the infectious material in the remainder. Both processes are easy to audit for enforcement purposes. It is not possible to remove all